

# What electronic future for Europe?

The European Commission is disappointed that past investment in research and development has not yet made European electronics flourish. It may be over-impatient, but it also needs a better strategy.

GOVERNMENTS seeking to stimulate what they call information technology have usually seen their investments run into the sand. The French government's schemes for keeping IBM at bay, in the 1970s, achieved much less than intended. So did the British government's Alvey programme in the early 1980s. Those salutary examples should be some comfort in Brussels, where the support of the European Commission (EC) for modern electronics and its applications is now being looked at more quizzically than for several years. The EC's research managers might even console themselves that the rules of international trade, which forbid the direct subvention of particular industries or companies except in predefined circumstances, are almost calculated to ensure that their programmes must fall short of providing the research and development base for an internationally competitive electronics industry. But that may be only small comfort.

The EC's love-affair with electronics goes back to the late 1960s, when M. Pierre Aigran, then head of the French government's research directorate, urged on Brussels specific support for telecommunications and computer technology. It was not until 1980 that the European Commission was given the authority and funds to embark on its Esprit programme, which provides central support (usually on a 50:50 basis) for transnational collaborative research and development in electronics. (There is a similar, but smaller, scheme known as Brite for the support of the broad-band telecommunications system on which Europe had set its heart by 1993.) Although the quality of the work stimulated by Esprit has not been uniformly high, much of it is excellent, giving those involved much greater competence in their daily business.

## Failure

So where is Europe's bright new electronics industry? One of Europe's supposedly strongest companies (Philips of Eindhoven) has been discovered this year to have over-spent itself as, last year, had Germany's Nixdorf, while ICL Ltd, the residuary legatee of splendid British initiatives going back to the 1940s, is about to be mostly sold to its Japanese technical partner Fujitsu. No wonder there are second thoughts in Brussels.

High time. But will the Commission now come to the correct conclusions? There are two outwardly very different models of success in this field — the United States

and Japan — but in the breathtaking development of computer technology in the past decade, most of the running has been made in the United States. The explanation has three parts. First, at least three technically strong chip-making companies have fought with technical innovations for shares of a growing market. Second, multitudes of commercial Don Quixotes have tilted at the wind-mill of IBM, and many have survived, creating a huge and previously non-existent market for computer machinery, to the general benefit of the US economy. Third, a host of software companies, some of which have also survived, have set up shop, enormously cheapening the cost of putting the new technology to work. The revolution, and such it is, has been driven by the opportunities of an exploding market and sustained by the ingenuity of a generation of engineers bent on making a fortune for themselves. That does not sound much like Esprit.

## Flair

The Japanese contribution is not as different as it may seem. The outstanding flair of Japanese companies, the quality and reliability of their products apart, is the speed with which they are able to put innovations onto the shelves of the electronics shops. The companies seem like the well-ordered and centrally directed corporations with which Europe is all too familiar, but they also provide a framework within which eager innovators can win their bosses' financial and marketing support. (That the innovators' rewards may often be no more substantial than a golf-club membership may puzzle people in the United States, but that is a not entirely obscure cultural difference.) That, too, is not the Esprit model.

How should Europe, and the European Commission, respond to these events? The economic benefits of information technology are of two kinds: networks of small computers are ways in which people's capacity to do intellectual work can be magnified just as the steam engines of the late eighteenth century augmented the capacity of people's muscles, while computers and their ancillary equipment can be a source of profit to those who manufacture them successfully, or competitively. In striving for benefits of both kinds, the Commission may get neither.

The Commission's strategy, if that is the right word, has been its determination that indigenous European manufacturers should not be absent from among the world's suppliers. That underlies the Esprit programme and ex-



plains the Commission's support for the JESSI project (from which Philips has now withdrawn), intended to create substantial European chip-making competence. But the Commission has never made clear what would happen if JESSI proved technically successful but commercially uncompetitive with, say, Intel Inc. of the United States. Would European manufacturers then be protected from competition by even higher tariffs, at the expense of Europe's potential users of information technology and thus of Europe's competitiveness?

As events are now unfolding, and with the prospect of a single European market, what the Commission should most of all be concerned with is that European business should be ready to support technical innovation, even relatively minor innovations, generously and decisively. That has more to do with the behaviour of the banking system and the stock markets than with programmes of direct support of research and development that must, by definition, be 'precompetitive' in some sense not clearly defined. Certainly there can be little good sense in seeking still to encourage transnational collaboration as if it were an end in itself when national economic boundaries will have been swept away. But there is also a need for greater numbers of young Europeans willing to take their chances as innovators, which is why the Commission should be more concerned with higher education and research than its member governments have so far allowed. A reappraisal of the EC's support for information technology should edge it in that direction. □

## Copyright unlimited

One of the casualties of the budget wrangle in the US Congress may be a sensible reform of US copyright law.

WHEN is publicly available written material not to be counted as a publication? When it consists of a great personage's private papers deposited in a publicly accessible library, often in return for substantial sums of cash. The issue was dramatized two years ago, when the author J. D. Salinger legally prevented the publication of a biography based on his private correspondence deposited at Austin, Texas. That is one reason why Senator Orrin G. Hatch has been labouring for months, with a committee of the US Senate, to secure an amendment of copyright law. But in the closing weeks of the present Congress, the reform has lapsed for lack of time. One of the issues raised has been the fear of computer manufacturers that unpublished computer codes incorporated in computers put on sale would also be robbed of protection by the planned amendment.

The issue is intricate. Copyright law makes possible the publication of books, journals and other creative works (maps, for example) by giving their publishers a right to prevent others from copying what they put out. Otherwise, authors and their publishers would regularly find their best-sellers pirated. But copyright in written material not formally published (and so not deposited at a

copyright library) remains the perpetual property of the author or of his heirs and assigns, and such material may not be published without consent. Those sitting on a sheaf of racy correspondence from, say, a distinguished Nobel prizewinner should not think that they can quickly turn it into a bestseller. That is also as it should be. Private correspondence would otherwise quickly dry up. Yet is there not a case that material voluntarily deposited at a public library by an author or, after death, by relatives, often in return for quite substantial sums of money, should be regarded differently? That was the question underlying the proposed Hatch amendment.

The question demands an answer, one less wooden than in present practice. Great personages' papers are presumably deposited on public access at libraries so that scholars may form a better understanding of a life's work. What sense can it then make that the same scholars should not be able to illustrate what they may write with quotations from the publicly accessible unpublished material? There may be a case for restricting this right during an author's lifetime, and for requiring that those who exercise it should be responsible, for example, for libels against persons still alive even in quotations from authors long since dead. But that would be better than the present arrangement.

Why the computer people should be alarmed is mystifying. Ostensibly, they fear that a relaxation of the rules on written copyright would hazard copyright protection of undeclared software simply because, being binary, it cannot be published in the ordinary sense of the word. In the present climate, in which the United States is slightly paranoid about the theft of US intellectual property, the argument carries weight. But the circumstances are so different from those affecting written materials on library deposit that the distinction can surely be made watertight in law. What else are lawyers for? □

## Agony continued

Where will the continuing contraction of the British research enterprise next strike?

LEST it be thought that the contraction of research in Britain has ended — or that there is no more left to cut — it should be known that a further 61 posts are to be cut from the establishment of the three horticultural research institutes maintained by the Agriculture and Food Research Council (AFRC). The explanation is familiar — a static research council budget, increased costs and a laudable ambition to make more research grants to academics. The AFRC has borne the brunt of contraction in the past ten years, chiefly because of the Treasury opinion that research is pointless when agricultural surpluses are a European embarrassment. Will the Medical Research Council be next to suffer, on the grounds that, with unemployment rising and the British transport system overwhelmed by traffic, there is plainly now also a surplus of people? □



# Science defeats all odds in US budget

- Increases set for science agencies
- NSF budget doubling back on target

## Washington

WHILE the US Congress continued its eleventh-hour assault on the federal budget deficit last week, science agencies were already breaking out the champagne. Despite grim predictions of across-the-board cuts, research funding is likely to outscore every other federal spending category with average increases of nearly 12 per cent likely in the final 1991 budget.

Two of the four top science agencies did even better with increases of about 13 per cent set as the House of Representatives and the Senate agreed on a compromise between their budget figures. The good news for the National Institutes of Health (NIH), the National Aeronautics and Space Administration (NASA) and the National Science Foundation (NSF) was a payoff that agency officials attribute to a year's concerted lobbying by scientists and their research advocates.

The NIH reaped much of the benefit with an increase that is almost 10 per cent over last year, and about 5 per cent over President Bush's request in January. Total NIH funding, including training and AIDS research, will be \$8,307 million in 1991. Controversial language calling for a four-year NIH spending plan which, among other things, would favour universities with low overhead costs (see *Nature* 347, 413; 4 October 1990) was retained in the conference report, and will effectively become law. Ten committees assembled by NIH will be deciding this week how to implement the language, so that NIH can report back to Congress within 30 days.

Buildings and facilities at NIH will get over \$1,000 million in new funds. That increase, 177 per cent over last year's appropriations, will largely go towards a child health/neurosciences building and a large office building on the NIH campus.

Other NIH highlights include the human genome project, which ended with just under \$88 million, somewhat better than the \$66 million the House had threatened earlier this year, but still far below the requested \$108 million and a disappointing (47 per cent) increase for a new project that was meant to double in size. Elsewhere in the US health budget, the Centres for Disease Control received an increase of nearly 18 per cent to \$1,318 million and the Alcohol, Drug Abuse and Mental Health Administration won a 10 per cent increase to \$2,895 million.

Overall, research advocates could find little not to like in the congressional numbers. "Biomedical research is extremely

popular in Washington. Scientists worked hard to get that across and the message came through," says Gar Kaganowich, head of public affairs for the Federation of American Societies for Experimental Biology.

NASA, the largest US science agency, also did well, although it was only about half the ambitious 24 per cent rise President Bush had asked for in January. Total NASA funding is \$13,868 million, \$1,646 million over last year.

The Space Station and the Moon-Mars mission were the only major projects to be hit. In addition to cutting the president's budget request for the Space Station by \$551 million to \$1,900 million, the House-Senate conference put a 10 per cent (including inflation) cap on any future rise in the starcrossed project's total cost. The conference report also requires that NASA re-examine the entire project. That process, which will take the form of a 90-day study, will begin immediately, according to a NASA spokesman. The impact of the 22 per cent cut in next year's funding will not be determined until the study is completed, NASA says.

As expected, Congress entirely deleted the Moon-Mars mission from the budget, for a saving of some \$300 million. On the plus side, the House-Senate conference restored all but \$3 million to the \$148

that NSF had hoped to start this year. Although \$75 million was deleted at the last minute from NSF's research request (Congress discovered that it could not make the military pay for the operation of NSF's Antarctic scientific stations), eliminating LIGO leaves only \$28 million in cuts to be spread out among all NSF's other science programmes. "I don't think it will mean any restructuring of priorities", says NSF budget officer Joel Whitter.

DOE science programmes fared less well in last week's congressional conferences. Although overall figures are up by about \$300 million — some nine per cent — the inflation rate is expected to rise by nearly that much as a result of a general slow-down in the US economy and the Middle East crisis. Within the DOE programmes, Congress cut \$50 million each from the budgets for magnetic fusion (which puts it 15 per cent below 1990) and general research. A DOE spokesman says that the agency has not yet decided whether to make a percentage cut across the board in those programmes or to target specific projects for reductions.

The Superconducting Super Collider (SSC) lost \$75 million from its \$318 million request but the conference report allows it to use \$25 million in 1990 construction funds that had been frozen in congressional action last year. Late last week, the Texas National Research Laboratory Corporation, which manages the \$1,000 million that the state of Texas has promised the project, voted to release \$60 million of its funds to more than make up for the remaining shortfall. The end result, says SSC director Roy Schwitters, is that the congressional cut will have "zero impact" on the project.

Elsewhere, Congress cut the Strategic Defense Initiative ('Star Wars') budget by \$1,800 million to \$2,900 million, the lowest level in five years, and a sign that the controversial programme may be near the end of its days. And in the transportation budget, Congress approved \$10 million in funding for research into US designs for magnetically levitated (maglev) trains, a technology that, based on superconducting magnets, has been under development in Japan for almost a decade.

Although Congress is expected to remain embroiled in budget negotiations throughout the week, the figures from last week's conferences are unlikely to change greatly before final congressional and presidential approval. "Right now it's a self-contained fight within the revenue [taxes] side," says NSF's Whitter. "Assuming those talks don't break down completely and make them turn to spending, these numbers should be pretty solid." President Bush has given Congress until today to complete the budget, although an extension (which would be the third this year) remains possible.

Christopher Anderson

US SCIENCE BUDGET (\$ million)

|      | 1990   | 1991*  |
|------|--------|--------|
| NIH  | 7,450  | 8,307  |
| NASA | 12,221 | 13,868 |
| DOE† | 3,379  | 3,676  |
| NSF  | 2,080  | 2,356  |

\* Budgets approved by House and Senate conference

† General Science and Energy Supply R&D

million CRAF/Cassini paired cometary exploration probes. The Senate had previously recommended a \$50 million cut in the programme, which would have effectively cancelled CRAF (see *Nature* 347, 215; 20 September 1990). But heavy last-minute lobbying by planetary scientists apparently found a receptive audience.

Congress found far less to quibble with in NSF's budget. It granted the agency nearly all of its 1991 request, and for the first time in three years put the agency nearly on schedule for the long-promised doubling of its budget within five years. The only major cut was of almost all funds for the Laser Interferometer Gravitational Wave Observatory (LIGO), a project



# Quark-hunters are rewarded

## Washington

THE Nobel physics prize committee this year took what may be the final step in its curiously unchronological recognition of the experimental discoveries that have established, over the past few decades, the generally accepted standard model of elementary particle physics. The winners, Jerome Friedman and Henry Kendall of the Massachusetts Institute of Technology and Richard Taylor of the Stanford Linear Accelerator Center (SLAC), collaborated in 1967 on electron-proton scattering studies at SLAC that demonstrated the existence of smaller particles inside the proton. Murray Gell-Mann, a co-inventor with George Zweig of these subparticles, named quarks, won the physics prize in 1969, but, for reasons the secretive Swedes are unlikely to reveal, it has taken another 21 years for the experimental work that substantiated Gell-Mann's hypothesis to be similarly rewarded.

Although the results obtained by Friedman, Kendall and Taylor turned out to be of fundamental importance, their experiment was originally seen as a long-shot at best, a waste of accelerator time at worst. When it came on-line in 1966, the Stanford Linear Accelerator was mostly devoted to studies of elastic electron scattering, in which the aim was to record the deflections and energy losses of electrons bounced off protons. Inelastic scattering experiments, which Friedman, Kendall and Taylor decided to pursue, were referred to by some as "beam surveys": an electron was slammed into the protons at high energy to create a shower of new particles, and the debris was examined to see if any useful secondary beams (of muons, for example) could be extracted.

The standard wisdom at the time was that the mess of debris created by inelastic scattering was too complex to be understood in a way that would shed light on the inner structure, if there were any, of the proton. Elastic scattering, the modern version of Rutherford's famous experiment in which he scattered alpha particles off atoms to show the presence of small, dense, electrically charged atomic nuclei, was instead thought to be the way to probe the proton. But the first round of results from elastic scattering experiments showed nothing of interest, indicating only that the proton seemed to be a smooth, structureless distribution of charge. In retrospect, this was inevitable. Elastic scattering could have picked up the existence of a hard core to the proton, as in the Rutherford experiment, but charged quarks moving rapidly about inside the proton would, to the passing electron, be indistinguishable from a smooth charge distribution.

Inelastic scattering produced, as expected, a complex mess of new particles, but Friedman, Kendall and Taylor found that the statistical properties of this mess behaved in a relatively simple way at higher energies. This 'scaling' of the results of deep inelastic scattering (deep because the more energetic electrons were able to penetrate further into the proton) did not have any immediate interpretation, but it struck some theoretical physicists, in particular James Bjorken and Richard Feynmann, as a clue to some underlying simplicity in proton structure.

Feynmann realized that scaling made sense if, at high energies, the electrons were interacting with individual subunits of the proton rather than with its constituents as a whole. He called the subunits partons, and argued that the results of deep inelastic scattering showed that the partons moved rather freely inside the protons. This seemed to be at odds with Gell-Mann's theory, because his quarks had to be strongly interacting. At the same time, according to Bjorken, there were doubts about the reality of the scaling laws, and also alternative explanations for them that involved not proton substructure but new dynamical effects in the electron-proton interaction.

The programme of deep inelastic scattering studies was to continue for many years, accumulating results that allowed different explanations to be distinguished. On the theoretical front it was eventually realized that, at sufficiently high energies, quarks could theoretically behave at close range as if they were free particles, but still be inextricably bound up inside the proton.

By the mid-1970s, quarks had become real particles, not abstractions. And in the long run the success of the quark model put physics on the road towards unification mania, the goal of which is to assemble all the forces of nature into some single, all-encompassing model.

This year's physics award, in conjunction with that of two years ago, when Leon Lederman, Melvin Schwartz and Jack Steinberger were made Nobel laureates for their discovery of neutrinos in the early 1960s, corrects an omission that elementary particle physicists had long noted. In contrast, the 1983 discovery of the W and Z particles, crucial to unification of the weak and electromagnetic forces, was promptly recognized when Carlo Rubbia and Simon van der Meer won the physics prize the following year.

With most notable particle discoveries now rewarded, it may be that physicists will have to find the top quark or the electroweak Higgs boson before the Nobel will come their way again.

David Lindley

# Corey the logical choice

## London

THIS year's Nobel prize in chemistry has been awarded to Elias Corey of Harvard University, Massachusetts, for his contributions to synthetic organic chemistry. Corey is widely credited by chemists as having played a key part in transforming organic synthesis from something resembling a 'black art' to a discipline founded firmly on logic. That he has won the prize alone rather than sharing it is itself a testament to his position in the field and the pervasive influence of his ideas.

Over the past three decades, Corey has devised synthetic routes to more than 100 natural products, many of which have found wide use in medicine and industry. He is perhaps best known for leading the group that in 1969 made the first synthetic prostaglandins, molecules involved in the regulation of among other things blood pressure and the heart. But it was not so much his prolific output that attracted the Nobel Committee as his strictly logical approach to complex syntheses.

In the 1950s, at the start of his career, organic synthesis was largely a trial-and-error pursuit. Progress was being made in understanding the mechanisms of organic reactions and in developing useful reagents, but synthetic chemists still analysed target molecules case by case.

In the absence of general rules, the prevailing approach was to identify a possible starting subunit (a readily available reagent, for example) within the structure of the target molecule. The problem then became how to manipulate the subunit so as to generate the full structure.

This approach led to some notable successes, such as Robert Woodward's heroic synthesis of chlorophyll, one of the achievements that earned him the Nobel prize in chemistry in 1965. Yet by and large, chemical manipulations were still deployed in an *ad hoc* fashion, a practice that remained the norm until the 1960s when Corey began working on sesquiterpenes, a family of natural products whose structures were void of obvious starting subunits.

Corey reasoned that to devise logical synthetic routes to such molecules, it was necessary to work backwards from the end product, disconnecting chemical structure until one obtained a set of simple precursors. In seminal papers published in 1967 and 1968, Corey wove his ideas into a general synthetic strategy, coining the term 'retrosynthetic' analysis to describe it.

Put crudely, the analysis involves subjecting the target molecule of the synthesis to a series of stepwise dissections, according to a set of simple rules bearing on the

character and structural context of the chemical bonds being broken. Ultimately the analysis generates a 'tree' of possible synthetic intermediates.

The obvious advantage of the approach was that it made synthesis systematic. Nevertheless, it was only after Corey had successfully used it to synthesize a host of natural products that it became widely accepted. Now it is standard laboratory practice. "No one embarks on a synthesis these days without applying the retrosynthetic method", says John Mann, reader in organic chemistry at the University of Reading.

Corey was also quick to realize the potential power of the computer as an aid to analysing the hundreds of intermediates that can result from a retrosynthesis. In recent years, programmes have emerged that not only generate intermediates but offer guidance on picking the simplest route through them to the target

#### AUSTRALIAN UNIVERSITIES

## First strike over pay and conditions

Melbourne

Australian university and college staff last week held their first one-day strike in a campaign to protest against poor career structure and to demand wage rises. The 4,500 academic staff who went on strike warned that unless their wage claims were met by 1 November, they would take further action including a refusal to release examination results or enrol students.

The three unions involved, the Federation of Australian University Staff Associations (FAUSA), the Australian Teachers Union and the Union of Australian College Academics, representing 35 colleges and universities, want average wage increases of 17 per cent in order to make salaries competitive with those in private industry and at universities elsewhere. The increase would also bring academic salaries into line with the increased rates paid to scientists at the Commonwealth Scientific and Industrial Research Organization (CSIRO).

According to Di Zetlin, general secretary of FAUSA, wage increases are necessary to attract the academics needed to replace the large number who will retire in the next decade. "With an expected shortfall of 10,000 academics by the year 2000, the bottom line is that we must create more attractive salaries and career structures. Most of those retiring will be senior academics."

The unions are seeking a common salary scales and career structures for all academics in Australian tertiary institutions, an increase in tenured employment in universities, and a removal of barriers to promotion within the different salary ranges.

In October last year, the federal cabinet agreed to support an average award in-

molecule.

Born in 1928, Corey received his bachelor's degree from the Massachusetts Institute of Technology in 1948 and doctorate there two years later. From 1951 to 1959 he rose through the ranks at the University of Illinois before moving to Harvard where he has been ever since. He is the sixth Harvard professor to win the prize in Chemistry, and his award brings the total number of Nobel laureates on the Harvard faculty to 18. It is also the latest in a run of awards to Americans.

Finally, for scientists who consider teaching to be the bugbear of academic life, this year's chemistry prize holds a moral: it was Corey's quest for a more logical way of teaching organic synthesis that originally shaped his thinking about the subject. He has been much quoted since for maintaining that teaching and fundamental research are two sides of the same coin.

David Concar

crease for academics of 6.7 per cent. But CSIRO scientists, traditionally receiving salaries equivalent to those given to academics, obtained increases of between 5 per cent and 10 per cent above the cabinet allocation.

According to Simon Marginson, research officer for FAUSA, career structures also need to be reviewed. Junior academics are hardest hit, with fewer than one in ten in permanent employment, and many working on a casual basis without benefits. FAUSA is seeking increases in the number of tenured positions open to junior academics.

The Australian Higher Education Industrial Association (AHEIA), representing the employers, admits that there is both a case for a "major upgrading in salaries", and for a "greater proportion of staff having tenure at junior levels", according to Professor David Penington, president of AHEIA.

The biggest obstacle to negotiations between the unions and employers now seems to be the employers' demand that salary rises be linked to performance. In the present system, academics are assessed only when applying for promotion to a higher grade. Movement and salary increase within each grade, however, is automatic. The universities are trying to introduce a "soft bar" which can prevent a person progressing within a group if his or her work is not up to standard. Marginson sees this "soft bar" as "managerial interference and a way to delay the promotion of academics". Penington, however, points out that "all CSIRO's officers are continually assessed at every level. If the unions want us to follow the CSIRO system, then they must be prepared to undergo performance appraisal".

Tania Ewing

#### ASTRONOMY

## Another pulsar bites the dust

São Paulo & Washington

THE source of periodic brightness variations that led a team of Brazilian astronomers to announce that they had at last found a pulsar in the remnant of supernova 1987A was traced, a few days after the claim was made, to mechanical vibrations in the telescope. This is the second spurious 'pulsar' to have been found in SN1987A; the first, pulsing at a barely credible frequency of 2,000Hz (*Nature* **338**, 234; 1989) was eventually ascribed to stray emissions from nearby electronics. The latest non-pulsar was more short-lived, but the rumours were substantial enough to be noted in these pages (see *Nature* **347**, 511; 1990).

The Brazilian team, led by J. E. Steiner of the University of São Paulo, asked the Center for Astrophysics (CfA) in Cambridge, Massachusetts, to transmit an International Astronomical Union (IAU) circular to alert the astronomical community of their discovery. But according to Daniel Green of the CfA, the details were not wholly convincing, and a request for more information was sent to Steiner; he responded by asking for the IAU circular not to be sent, as unspecified problems had turned up.

Although the Brazilians, who made their observations on a 1.6-metre University of São Paulo telescope, checked their instrumentation by ascertaining that stars near the supernova remnant showed no pulsation, they later realized that their photometric detector was being confused by light from the nebula around the supernova site, and sent into some kind of instability. More negative evidence came from the European Southern Observatory (ESO) in Chile, where H. Ögelmann and C. Gousses were also looking for a pulsar. On the night of 28 September, when Steiner's team believed it had seen something, they found no optical pulsations.

John Danziger, an astronomer at ESO headquarters in Munich, says there is solid evidence for a source of energy in SN1987A in addition to the still-decaying radioactive debris of the explosion: its luminosity has been falling less rapidly than the simple exponential curve that radioactivity alone would produce. Energy coming from material falling onto a compact central object such as a neutron star can reasonably explain the observations, but the case that this is indeed a pulsar is only inferential. The excess brightness is entirely at infrared wavelengths, suggesting that energy from within is being reprocessed by the surrounding cocoon of dust, concealing the source from direct observation.

David Lindley



# Japanese plan to clean up

## Tokyo

TORN by disagreement between the powerful Ministry of International Trade and Industry (MITI) and the much smaller Environment Agency, the Japanese government on Tuesday adopted an "action programme to arrest global warming" that contains two different targets for limiting Japan's carbon-dioxide emissions by the year 2000.

MITI favours holding per capita carbon dioxide emissions to "about" this year's level by the year 2000. Because population will increase over the decade, this actually allows total carbon dioxide emissions to rise by about 5 per cent. The Environment Agency is backing the more optimistic goal of holding total emissions to the 1990 level and so requires a reduction in per capita emission levels. Whichever target is finally adopted, fairly drastic action will be required.

Saburo Kato, director of the Environment Agency's global environment division, says that he is "very happy and very proud" of the action programme's ambitious targets. In addition to reaching a compromise with MITI, success required the coordination and support of many other ministries and agencies including the Ministries of Transport and Construction, the Science and Technology Agency, the Ministry of Foreign Affairs, the Ministry of Agriculture, Forestry and Fisheries and the Forestry Agency. Kato stresses that the programme represents the consensus of all of these organizations and thus is "very deep and profound".

Apart from placing limits on carbon dioxide, the programme calls for methane levels to be held to their current level and for nitrous oxides and other greenhouse gases to be held to current levels "as far as possible". The plan covers the period from 1991 to 2010 and will be reviewed "flexibly" in response to international trends and scientific findings.

To meet the limits on carbon dioxide emissions various measures will be adopted. Areas of trees and greenery will be expanded in urban areas. Use of insulation, solar heating, waste heat (from sewage, subways and waste incineration) and co-generation of electric power will be encouraged to conserve energy.

Transportation systems will be renovated to encourage greater use of mass transit and to eliminate the problems of traffic jams in major cities like Tokyo. The development of fuel-efficient cars, electric cars and other energy-efficient transport systems will be promoted.

Energy conservation will be further encouraged in the manufacturing, agriculture, forestry, fishery and construction industries, even though this is an area in which Kato feels Japan "already leads the

world". And carbon dioxide emissions in the power-generation sector will be held down by developing nuclear power, increasing the use of liquid natural gas, and by introducing dispersed small-scale power sources such as fuel cells and photo-electric cells.

Finally, the general public in Japan will be encouraged to reduce carbon dioxide emissions by recycling products, by eliminating excessive use of packaging, vending machines, and direct mail, by reducing working hours and by encouraging everyone to take a summer holiday.

No laws have yet been introduced to implement the programme, but Kato says that emission levels will be monitored annually by all the various ministries and agencies under the coordination of his department to ensure that the targets are achieved. Tax incentives "may" be introduced, he says, to ensure adoption of some measures. But he is confident that the programme will attain its goals.

He points to the case of pollution control in Japan where industry initially opposed restrictions but eventually developed sophisticated pollution-control technology now being sold worldwide. Kato claims that all the ministries and agencies believe that the targets can be achieved "without any significant economic impact", even though MITI objected to the more ambitious target on the grounds that it would hurt the economy.

Whichever target Japan eventually attains, it should end up with one of the

## RESEARCH QUALITY

### David, meet Goliath

#### Washington

THE 220 researchers sponsored by the Howard Hughes Medical Institute (HHMI) are about as productive as the 3,000 scientists who work for the US National Institutes of Health (NIH), at least when it comes to "hot papers", according to the Philadelphia-based Institute for Scientific Information (ISI). In a study to be published in *Science Watch* later this month, ISI found that of the 894 most cited biology papers since 1987, HHMI investigators accounted for 82. Within NIH, on the other hand, more than ten times as many researchers managed to score a total of only 84 of those papers. In their defence, officials at the NIH say that their scientists have important clinical duties that HHMI investigators can safely ignore. Government scientists are also forced to endure government salaries and budgets. But the startling ISI statistics show that the HHMI has made good its intention to find and fund the best US scientists, even if it means shaming the NIH in the process.

Christopher Anderson

lowest levels of carbon dioxide emission in the developed world on a per capita basis. According to figures for 1988, from the Organization for Economic Co-operation and Development, only Italy and France have lower per capita emissions (2.01 and 2.04 tonnes of carbon per person per year, respectively) than Japan (2.45 tonnes per year). Japan's emission levels are already considerably less than the United Kingdom (2.97 tonnes per year), West Germany (3.45 tonnes) and the United States (6.14 tonnes).

It is hard though to see how many of the measures advocated in the current action programme will be implemented. Greening of cities and improvement of road transportation can be achieved only if land is available for parks and roads. That would require sky-high land prices to be brought down, something which the government has already shown itself incapable of doing. Measures being considered to reduce land prices, such as increasing taxes on farmland in urban areas, would actually decrease the already limited green areas. Furthermore, the Japanese stock market is propped up by the inflated value of land assets of companies and any drastic downward revision of land prices could cause economic chaos.

Similarly, the electric power industry will fight tooth and nail to prevent introduction of dispersed sources of power such as solar power and fuel cells that would reduce its monopoly. And even persuading the Japanese to take summer holidays will prove difficult because of the enormous social pressure to stay at work.

David Swinbanks

## AIDS RESEARCH

### NIH digging in

#### Washington

THOSE still optimistic that the war against AIDS will soon be over need only look to the US National Institutes of Health (NIH) for a reminder of the long haul ahead. On the assumption that no end is in sight, NIH this month announced that they were transferring more than \$2 million of their AIDS research funds into training grants to encourage about 60 new scientists to join the field. "The magnitude of AIDS and HIV infection will continue to grow for the remainder of the century", said Health and Human Services secretary Louis Sullivan in a statement. "These grants will help to expand the number of talented investigators . . . who can perform the research that will help to conquer this devastating disease." The funding, which will be distributed to postdoctoral fellows in 14 US universities and research institutes, is the first such effort to ensure that there will be a continuing supply of researchers in the years to come, says NIH programme director Gregory Milman.

Christopher Anderson



## HUMAN GENOME

## A different approach

### Paris

JUST two weeks after the official start of the massive US programme to sequence the human genome, France has decided to boost its own research efforts with the aim of garnering efficiently many of the fruits of research on the human genome. "We will eat the chocolate without the bread", is how Philippe Kourilsky of the Pasteur Institute describes the decision to concentrate on the 5 per cent of the genome known to code for proteins. To speed up the process, a four-year Anglo-French Eureka pre-competitive technology research project, Labimap 2001, aims to develop and market a series of machines to automate time-consuming sequencing operations.

According to research minister Hubert Curien, the estimated FF150 million (\$29 million) the state currently spends on genome research each year is "insufficient and too dispersed to support international competition". After consultation with leading French researchers, the Ministry of Research and Technology has decided to set up a new public body with its own scientific advisory committee, to distribute an extra FF50 million next year and FF100 million in 1992. "We do not intend to use this money for construction", says Curien. Instead, the grants will go to some of the 500 researchers and 67 laboratories already engaged on genome research. The main role of the new body is to coordinate the national effort, ensure international liaison and encourage the commercial exploitation of discoveries.

Curien is openly critical of the "messianic" declarations of James Watson, director of the US National Institutes of Health human genome research project. Last year, Watson warned that if the international community did not share the costs of sequencing the human genome, it would be denied access to the data. This challenge is "unparalleled in the medical and biological sciences", says Curien.

France has already played a key part in human genome research, through its centre for the study of human polymorphisms (CEPH), set up by Jean Dausset, a Nobel laureate, and Daniel Cohen in 1984. With a FF40 million annual budget (mostly state-funded but also supplemented with grants from the US National Institutes of Health and the Howard Hughes Medical Institute and national charities), CEPH makes available to researchers throughout the world a unique bank of genetic material from over 60 very large families. The aim is to use standard material more efficiently to localize and sequence the genes responsible for the 4,000 known hereditary illnesses." **Peter Coles**

## CONTRACEPTION

## Equality for the sexes?

### London

A MALE hormone-based contraceptive moved one step closer to practical reality last week with the announcement in *The Lancet* that pregnancies have been successfully prevented in the sexual partners of men receiving weekly injections of the hormone testosterone enanthate.

Earlier studies had already suggested that sperm production stops in a large proportion of men within a few months of their being injected with testosterone. But the new World Health Organization (WHO) study, spanning seven countries, is the first to test the supposition that these men become infertile. One hundred and nineteen men who appeared not to be producing sperm relied on the injections as their only method of contraception for 12 months. Only one of the men's partners became pregnant, suggesting that the male contraceptive is more efficient than female contraceptive pills (and far more efficient than the use of condoms).

The authors of the report indicate that even the single apparent failure may have been the result of an extramarital encounter. A small number of men withdrew from the study, complaining of acne or increased aggressiveness, but there was no sign of the more serious of the anticipated side effects, such as prostate problems, liver enzyme abnormalities and shrinking testicles.

Geoffrey Waites, who coordinated the study from WHO's base in Geneva, says the next step is to run a similar study on men whose sperm production has been much reduced, rather than halted. Six months of hormone injections completely abolished sperm production in only 65 per cent of the men, but laboratory tests suggest that the few sperm cells in the ejaculates of the remainder are "not functional", says Waites.

Finding an alternative to weekly hormone injections will be important if the treatment is to be accepted as a practical method of contraception. A male pill is unlikely, because testosterone is destroyed rapidly in the stomach, but Waites says it should be possible to achieve the same results with only three injections a year. WHO has developed a formulation of testosterone bound to esters which is expected to release the hormone steadily over a number of months, after an injection into muscle.

Pramilla Senanayake, from the International Planned Parenthood Federation, hopes the treatment will be generally available within five years, providing a realistic alternative to the almost universally disliked condom. Many Islamic people are also opposed to surgical techniques, such as vasectomy, Senanayake

says, but injections should be more acceptable.

WHO is also developing a reversible vasectomy technique, currently being tested in China. Small silicone plugs are injected into the sperm ducts, and can be removed in a relatively simple operation. But a 'contraceptive vaccine' for men, which attracted considerable media attention when the idea was first mooted, is still many years away. Paul Primakoff, from the University of Connecticut, has shown that male guinea-pigs injected with the sperm surface protein PH-20 are infertile (see *Nature* **335**, 543; 1988). But his group has since found that the treatment sets up an autoimmune inflammatory reaction in the testes, which Primakoff describes as "a clearly unacceptable side-effect" for a human contraceptive.

**Peter Aldhous**

## NUCLEAR WEAPONS

## US halts plutonium processing

### Boston

THE United States last week quietly abandoned its plans to continue to produce plutonium. Although the US government has often said that it has no shortage of plutonium, the change in policy owes more to concern about safety at plutonium production plants than either the existence of plentiful stocks of plutonium or the warming of East-West relations.

The Department of Energy will now reopen the the Purex processing plant, which since 1955 has separated plutonium for use in nuclear weapons from the spent fuel of nuclear reactors.

The Purex plant, located at the Hanford Nuclear Reservation in Washington state, was the only remaining US plutonium processing plant and was closed unexpectedly at the end of 1988 for safety reasons. Since then it has been the object of a lawsuit by a coalition of environmental groups. The plant had been scheduled to reopen this spring, but the Department of Energy now says it will leave the plant on "standby" and will prepare an environmental impact statement "to assess the likely effect of any future restart" of the plant.

The US Congress last week also cut funds for a new \$385-million plutonium-processing laboratory at the Los Alamos National Laboratory in New Mexico. The decision followed the cancellation by President Bush last spring of plans to build a plutonium-processing plant in Idaho, and a congressional decision in August to cut money for a plutonium-processing facility at the Department of Energy Rocky Flats installation in Colorado. **Seth Shulman**

# Disarray over declaration

## London

THE Second World Climate Conference in Geneva next week will be the scene of frenzied diplomatic activity, as government officials attempt to draft, almost from scratch, the first important international statement on climate change following the completion of the key report from the Intergovernmental Panel on Climate Change (IPCC). Environment ministers from almost 100 countries are expected to find large sections of the draft text still in dispute when they arrive for their meeting on 6–7 November.

Preliminary negotiations to draft the declaration in Geneva at the end of last month ended with deadlock over almost every important clause. Paul Hohnen, from Greenpeace, who attended last month's drafting meeting as an observer, says the atmosphere was one of "total chaos and confusion".

The declaration will not be legally binding, but is significant because it will set the tone for negotiations towards a climate change convention which will begin in Washington in February 1991. As expected, the United States, backed by Saudi Arabia, is fighting attempts by many European countries to include statements on the need to control carbon dioxide emissions. If some industrialized countries seem unwilling to negotiate preliminary emissions targets, Brazil and other developing countries will hold out strongly against the inclusion in the conference declaration of any firm statements on deforestation. Developing countries are also seeking assurances on technology transfer, and the establishment of a new climate fund, to help poorer countries tackle the climate change problem.

But the United States is worried that the proposed fund will become a 'bottomless pit', sucking in US money.

The ministerial meeting will be the focus of media attention in Geneva, but is preceded by a six-day scientific meeting beginning on 29 October. Indeed, the Second World Climate Conference was originally planned to review ten years of the World Climate Programme (WCP), the major international effort to co-ordinate climate research, set up in Geneva in 1979 at the First World Climate Conference.

In addition to the now-completed IPCC report, delegates in Geneva also have a new three-volume climate change report from the Stockholm Environment Institute (SEI) to consider. Jill Jäger, who heads the SEI climate programme, says the report is intended as a complement to IPCC, not an alternative. But she says that the SEI's approach differs from IPCC's by starting with a long-term environmental

target, and then defining the measures needed to get there.

The SEI report argues that the rate of global warming should be kept below 0.1 °C per decade, and that the increase in temperature should not be allowed to exceed 2 °C in total.

"Our target is based on what natural ecosystems can stand", says Jäger. The report argues that this target can be achieved cost-effectively through implementing large-scale programmes of energy efficiency and afforestation, and increas-

CANCER RESEARCH

# Uncharitable actions

## Washington

SOME of the largest US cancer research charities say that their fund-raising efforts this year continue to be dogged by 'look-alike' charities that spend most of their collections on themselves rather than on research. Such 'charities' may now be costing the cancer research community some \$15–\$20 million each year in lost revenue, says Michael Heron, a spokesman for the American Cancer Society (ACS), the largest US cancer charity.

Shielded by a loophole in US tax law, charities can operate with tax-exempt status for years without diverting more than a small fraction of their proceeds to the research, education or patient services they advertise as their purpose. "A few unscrupulous characters can poison the well for all us", by tainting philanthropy with deceit, Heron says. US charities support nearly \$120 million in cancer research annually but growth has slowed in recent years; in part due to increasing competition from new organizations, many of which spend most of their contributions on further fund-raising.

Some of the worst offenders may spend more than 100 per cent of their donations internally, according to the Virginia-based Council of Better Business Bureaus (CBBB), which tracks the performance of US charities. Defunct and often under investigation by local authorities or the Internal Revenue Service, such charities may fold, only to re-emerge later under another name and address — often uncannily similar to that of established cancer charities or research organizations.

Of the 11 charities tracked by CBBB, three — the Cancer Fund of America, the Walker Cancer Research Institute and the Pacific West Cancer Fund — "do not meet our standards", says communications director Margret Bower. In 1988, Pacific West managed to spend 112 per cent of the \$1.38 million it received on its own fund-raising (it finished the year more than \$800,000 in debt). Fund-raising

ing the proportion of energy that is generated from solar power and other 'clean' technologies — a package that the SEI estimates could reduce world carbon dioxide emissions by 40 per cent from 1986 levels by 2020.

But many governments will reject the SEI's optimistic analysis of the cost of reducing emissions, and also the strictly ecological criterion chosen to set the initial environmental target: concern over the uncertain effect of global warming on national economies may be a more powerful influence on the forthcoming negotiations than damage to natural ecosystems.

Peter Aldhous

consumed 70 cents of every dollar donated to the Walker Institute in 1988. And the Cancer Fund of America in the same year spent only 28 per cent of its \$7.76 million donations on actual cancer programmes, according to the CBBB statistics.

CBBB standards call for charities to spend no more than 35 per cent of contributions on fund-raising. Last year, fund-raising accounted for only 15 per cent of ACS's contributions. Other cancer charities that pass CBBB standards spend from 6 to 32 per cent of their income on fund-raising. A Cancer Fund spokeswoman says that fund-raising cost were admittedly out of control in the three-year-old organization's first two years, but "we're over the hump now". She claims 1990 figures will be close to CBBB standards.

Many of the charities identified by CBBB use similar tactics in soliciting contributions. In mass mailings, Pacific West and the Walker Institute sent out letters offering a 'sweepstake' with a 'free mystery gift'. In the letter, all recipients were notified that they had won a 'first round [cash] prize'. Closer examination of the letters showed that a minimum \$5 contribution was required to obtain the 'free' gift. In the case of a similar '\$5,000 sweepstake' offered by the Cancer Fund of America, the prize, for most, was a cheque for 10 cents.

All three charities tracked by CBBB are facing legal proceedings in several states, in some case for complaints of "unfair, unconscionable or deceptive methods, acts or practices". But local authorities cannot remove a charity's all-important federal tax-exempt status, which allows donors to write off contributions. Known as a 501(c), the provision is reviewed by the Internal Revenue Service only after it establishes a "spending history", which may take several years. By then, however, the charity may be history, leaving cancer research little, if anything, to remember it by.

Christopher Anderson



# Japan sees gold in warming

## Tokyo

WHEN it comes to developing new technology that has a prospect of selling well, Japanese industry shows no lack of enthusiasm for saving the global environment. According to figures released this week, more than 60 major companies and economic organizations have now agreed to contribute a total of ¥50,000 million (\$400 million) for the establishment of a new research institute for "environment-friendly technology" at Kansai science city, located close to Osaka, Kyoto and Nara.

The Research Institute of Innovative Technology for the Earth (RITE) is being established under the jurisdiction of the Ministry of International Trade and Industry (MITI) which is pouring over ¥70,000 million a year into the research and development of substitute chlorofluorocarbons, biodegradable plastics, and technology to reduce carbon dioxide emissions or to absorb and utilize the gas. In addition to the money from MITI and industry, local prefectural and municipal governments will inject another ¥30,000 million into RITE making the institute probably the best-funded national project ever in Japanese history.

RITE is due to open in 1992 on a plot of land provided free by Kyoto Prefectural government. In addition to providing ¥17,000 million of the ¥30,000 million in local government funding for RITE, the Kyoto prefectural government will also level the site for RITE, which is located in a steep valley, free of charge.

According to a list provide by Tsutomu Yamaguchi, senior managing director of RITE, 60 major companies covering almost the whole range of industry will be contributing money and researchers to the new institute. The participants include electronic companies such as NEC, Toshiba, Sharp, Hitachi, Sanyo and Mitsubishi Electric; the steel manufacturing companies Nippon Steel, Kobe Steel and Nippon Kokan; heavy industries such as Kawasaki Heavy Industries, Ishikawajima Heavy Industries and Hitachi Zosen, a shipbuilding company; chemical companies like Takeda Chemical Industries; the car manufacturers Toyota and Nissan; electric power companies; gas companies; and even banks, trading companies, insurance companies, and Japan's leading manufacturer of women's underwear, Wacoal.

Many of the companies involved are from the Kansai area and particularly prominent is the Sumitomo group, including Sumitomo Metal Industries, Sumitomo Marine and Fire Insurance Co., Sumitomo Chemical Co., Sumitomo Bank, Sumitomo Heavy Industries, Sumitomo Electric Industries and Sumitomo

Life Insurance Co. Keidanren and Kansai Economic Federation will also support the institute.

According to Yamaguchi, over 200 company researchers are already engaged in joint research with MITI's national laboratories and 80 researchers will be

## Voters' clear message on segregation

### Cape Town

PARENTS at two all-white high schools last week voted overwhelmingly in favour of opening the schools to all races. This is the first of many such polls to be held in South Africa in the next two months. That both schools are located in Uitenhage, an industrial town represented in parliament by a member of the Conservative Party, suggests the emergence of a new sense of realism in white communities.

The government is faced with over 300,000 empty places at white schools, and an estimated 1 million black children with no schools to attend. Reluctant to open white schools at all, the government has passed the buck to the parents, presenting them with a set of complicated options.

The first two options are to be 'privatized' (in which case the state pays the school a 45 per cent subsidy) and 'state-aided' (with the state paying a 75 per cent subsidy). In both cases, the school can determine its own admissions policy. The third option is to remain fully state-controlled and financed, either with open status (but with whites being given priority for places) or with whites-only status.

The first two options both require the approval of 72 per cent of a poll of at least

assigned to RITE when it opens in 1992. Yukinori Fujiwara of the Kansai Economic Federation says that, although banks, trading companies and insurance companies will not be directly involved in the research, they have joined because they realize that the global environment problem and measures to combat it will affect the whole of Japanese industry.

David Swinbanks

80 per cent of the parents, as does the third option if the school wishes to become open; only schools not wishing to do so are absolved from holding referenda. Even so, the final decision on the fate of each school lies with the Minister of Education and Culture, Piet Clase, and the poll results are merely intended to guide him in making decisions. This process should keep even his vast army of bureaucrats busy for many months to come.

Clase's plan cannot solve all the problems of the educational system because it does not address the severe shortage of teachers, particularly in science and mathematics. Last year in white secondary schools (which received the highest level of *per capita* funding), 36 per cent of physical science posts, 32 per cent of mathematics posts and 21 per cent of biology posts were occupied by persons with either no, or only one year of, university education in their subject.

Nor is there much hope for future recruitment of teachers. In 1988 South Africa's twenty-one universities produced only 223 mathematics, 176 chemistry and 102 physics majors between them, and only a fraction of them are likely to enter the teaching profession. Michael Cherry

## The taxman's human face

### Tokyo

THANKS to the Ministry of Finance, Japan has managed to find a way around the strange rules that forced Japanese recipients of grants from Japan's own Human Frontier Science Program to return half of their grants in tax. The problem began because the programme set up its headquarters in Strasbourg, meaning Japanese universities were forced to classify Frontier grants as 'foreign funds', even though most of the money originally came from their own government.

In April, when the first grants for research on the brain and molecular biology were given out, Japanese recipients were dismayed to discover that Ministry of Education, Science and Culture (MESC) regulations prohibited universities and other MESC-affiliated research organizations from receiving these 'foreign funds' directly. Instead, the researchers had to

receive the money themselves and then 'donate' it to the university. But that meant the grants were treated as 'personal income' and subject to 40–50 per cent income tax.

Officials of the Science and Technology Agency (STA) and Ministry of International Trade and Industry (MITI) tried to solve the problem by asking MESC to change university regulations. But MESC refused to cooperate because, as one MITI official put it, "they want to keep strict control over the universities".

MITI and STA then turned to the Ministry of Finance which last week gave the Frontier grants special tax-exempt status.

"Extensive efforts" and "vigorous" persuasion were needed, but in the end the "Japanese taxmen are finally found to be generous", says Ryuji Shimoda, director of STA's Frontier office.

David Swinbanks



## Jack of all trades

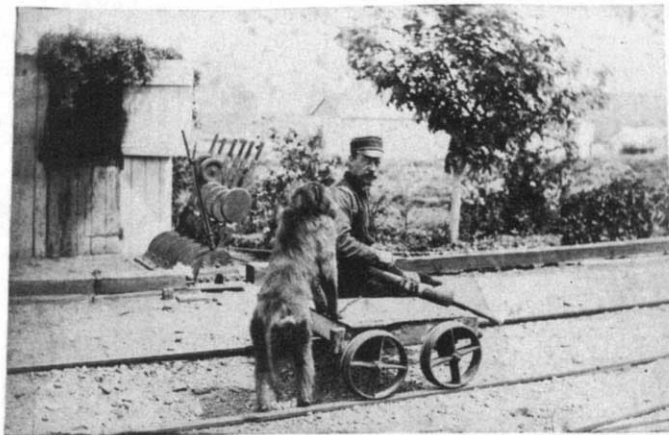
SIR—May I correct an error made in *Nature* 100 years ago — quoted from *Nature* 24 July 1890 in Then and Now of 26 July 1990? Your correspondent, J. F., mentioned a large ape said to be acting as a signalman on a railway in Natal. In fact, the ape — a baboon — acted as a railwayman at Uitenhage in the Cape, *not* in Natal. The story is of some interest and is well-documented.

Mr Wide, a railwayman, had lost both lower legs. He trained a baboon, Jack, to

account (of which I have a copy) is published in the *Cape Mercury* of 29 May 1923. It relies on an interview with Wide, the written statements of 25 witnesses, filed in the museum collection, and on Howe's evidence.

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Railway Jack, Wide and their trolley. Initially, Jack pulled the trolley by a chain attached to a hook on Wide's wooden legs, in tandem with a dog. Later the dog was killed by a train and Jack discovered it was easier to push the trolley. The trolley was set on the rails and taken off again by the baboon, twice daily. The control levers are in the background. Photo c. 1890, collection G. B. Howe.

work the lever of the signals and to carry out various other duties, including pushing Wide to work and back on a trolley which the baboon had to put on the rails (shown in photograph), pumping water, gardening and locking the door. On one occasion, when Wide had injured his arm, Jack took over all his signal-changing duties. According to a reliable witness, "Jack knew every one of the various signals and which lever to pull as well as his master himself". Not unnaturally, the railway passengers objected initially, but "the baboon never failed during his many years of work; and on several occasions he acted in a manner simply astounding to those who have not had personal experience of the high degree of intelligence possessed by these animals". Jack also defended Wide in a quarrel, and on another occasion drove off a foreman by beating him with a dirty coal sack.

Mr Wide and Jack were extremely close, a relationship expressed by mutual grooming. The story was documented originally by the Rev. George Howe, in 1890, and then by F. W. Fitzsimons, director of the Port Elizabeth Museum. Fitzsimons'

## The value of herbaria

SIR—As scientists familiar with the chemical and pharmacological study of plants at one of the major pharmacognosy research centres in the United States, we were appalled by the Commentary by Clifford *et al.*<sup>1</sup> advocating the dismantling of the world's herbaria. Many criticisms have been published in Correspondence<sup>2</sup>.

One of the most vivid examples of the value of general taxonomic collections comes from a study in the genus *Stevia* (Asteraceae)<sup>3</sup>. Stevioside, a non-caloric sweetener commercially used in Japan, is extracted from the leaves of the sweet-tasting plant *Stevia vebaudiana* (Bertoni) Bertoni. To determine whether other *Stevia* species contained this compound or related sweet-tasting compounds, we taste-tested small pieces of herbarium specimens of 110 species of *Stevia* housed in the collections of the Field Museum of Natural History to provide a comprehensive organoleptic survey for the presence of intensely sweet compounds in the genus.

Following the reasoning of Clifford *et al.*, this survey could have been performed by conducting field work to re-collect the 110 *Stevia* species based on the locality

information shown in records of label information: given the commercial interest in non-caloric sweeteners, such funding might have been obtainable. Were such a mission to have succeeded in re-collecting the species, it would have done so at considerable cost.

As it turned out, we found no additional species of *Stevia* with sweet-testing leaves. Disappointing? Yes, but how much more disappointing to have spent thousands of dollars to have obtained the same result.

Herbaria are vast biological libraries that house information whose future importance we cannot even begin to evaluate. The creativity of nature is much broader than that of humans, and we can ill afford to lose the results of nature's infinitely varied experiments. The folly of destroying libraries of books because they are expensive and because individual accessions are used only intermittently is obvious; the idea of destroying irreplaceable biological libraries is equally absurd.

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## Runaway complexity

SIR—Here is a teaser that I have never seen adequately addressed: what keeps simple organisms (such as bacteria) staying simple, while other lineages develop extraordinary degrees of morphological complexity? The problem: in general, the more complex the organism, the smaller the population size and therefore the smaller the number of potential selective deaths that can generate further complexity. Is the answer that more complex organisms throw up ever wider ranges of variation on which selection can act, despite smaller populations and ever fewer opportunities for selection events? Is there any particular predisposition in lineages that have 'evolved furthest' or have they just been subject to a sort of runaway complexity?

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Letters submitted for Correspondence should be typed, double-spaced, on one side of the paper only.

# Accountability in the greenhouse

Allen L. Hammond, Eric Rodenburg and William Moomaw

An international climate convention will require a means of measuring relative national emissions of greenhouse gases that is simple, fair and (preferably) empirical.

THE recently published report of the Intergovernmental Panel on Climate Change performs an admirable service in establishing a widely agreed scientific basis for discussions of greenhouse warming'. Now the action shifts to the diplomatic front. But the negotiation of a climate convention and subsequent monitoring and enforcement of a protocol for stabilization or reduction of greenhouse gas emissions will require a means of comparing relative national contributions to climate pollution.

The method used in the IPCC report is based on modelling the carbon and other biogeochemical cycles to calculate global warming potentials of the major greenhouse gases projected into the future for 20, 100 or 500 years. But this method, although appropriate for scientific purposes, is fraught with difficulties when considered from the point of view of international diplomacy (see, for example, ref. 2). The models are complex and still the subject of considerable scientific disagreement. Moreover, the different time periods yield significantly different global warming potentials because of the disparate lifetimes of the main gases in the atmosphere. The time periods used in the IPCC report are arbitrary; the diversity of results is a prescription for diplomatic chaos. Countries with high methane emissions will naturally prefer a longer time horizon which, because of methane's short lifetime, minimizes its effects; countries with high carbon dioxide emissions will prefer shorter time horizons. Disputes over the relevant choice could easily be used to delay international agreements, and hence action.

## New proposal

We propose a different approach, one that is empirical, yields relatively robust results, yet provides a simple and unambiguous basis for international agreements. It also lends itself easily to corrections as new data become available and, because it requires only a personal computer and a spreadsheet, is readily accessible to every country for policy analysis and planning purposes. Our method focuses on the instantaneous heating effect of greenhouse gas emissions (a time horizon of one year), rather than choosing an arbitrary time many years into the future, and on the incremental additions to the atmospheric burden of these gases in a given year. In effect, our

method ties observable current results directly to policy actions by assuming that, for any given year, the past is past and the future behaviour of the atmosphere is unknowable — a point of view that coincides more closely to a political than to a scientific world view, but which may be more appropriate for international agreements.

Direct measurements of gross emissions of greenhouse gases are not a useful measure of national contributions to the atmosphere's warming potential, as CO<sub>2</sub> and CH<sub>4</sub> participate in complex natural cycles with many sources and sinks. The observed increase in atmospheric CO<sub>2</sub> is only about half of the anthropogenic CO<sub>2</sub> emitted; for CH<sub>4</sub>, the increase is about one-fifth of the anthropogenic emissions, whereas essentially all the chlorofluorocarbons (CFCs) released to the atmosphere add to its heating burden. Thus emissions of different gases are of differing importance — quite apart from the infrared heating effectiveness of these molecules, which also differ. One way to take these differences into account is to model the relevant natural processes.

A second method, which we prefer, is to let the atmosphere itself sort out the natural sources and sinks, and to focus on the observed increase in atmospheric concentrations of greenhouse gases, which can be measured with relatively high precision. The increase in greenhouse gases in the atmosphere from year to year can easily be calculated, and gross anthropogenic emissions can be used to scale the observed increases. We do this separately for each gas and each country — in effect, allocating a portion of the observed atmospheric increase to each country in proportion to its share of gross emissions. When each gas is weighted by its infrared heating effectiveness and the contributions from each gas added, country by country, the result is an index representing each country's share of the observed increase in the heating potential of the atmosphere, in tons of carbon equivalent.

We have applied this approach to the four most important greenhouse gases (CO<sub>2</sub>, CH<sub>4</sub>, CFC-11 and CFC-12), making use of a unique set of estimates of national emissions compiled for 146 countries by the World Resources Institute (Table 24.1 of ref. 3). The approach could readily be extended to additional gases if emission estimates by country were available; even

so, we are able to allocate to specific countries about 85 per cent of the observed increase in the atmospheric heating burden, and the remainder is unlikely to significantly alter the country rankings that result. The observed increase in 1987 over 1986 was 1.7 parts per million for CO<sub>2</sub>, 17 parts per thousand million for CH<sub>4</sub>, 15 parts per 10<sup>-12</sup> for CFC-11, and 17 parts per 10<sup>-12</sup> for CFC-12 (Table 24.3 of ref. 3). Using a standard atmosphere, these increases in concentration imply increases of 3.7 thousand million metric tons of carbon as CO<sub>2</sub>, 48 million metric tons of CH<sub>4</sub> and 730,000 metric tons of CFCs. These empirical quantities are the additions of these gases to the atmosphere in 1987, net both of anthropogenic and of natural causes. It is plausible to attribute these additions to human activity, as concentrations were relatively stable (or zero, for CFCs) and the natural cycles were presumably in balance in the millennia immediately before the industrial revolution.

## Greenhouse index

The World Resources Institute estimates are that anthropogenic emissions of these gases in 1987 amount to 7.9 thousand million metric tons of carbon as CO<sub>2</sub>, 270 million of CH<sub>4</sub> and 730,000 of CFC-11 and CFC-12. Nearly 32 per cent of the CO<sub>2</sub> emissions arose from changes in land use, largely deforestation; the rest are from fossil-fuel combustion and cement manufacture. Methane sources include (in descending order) livestock, wet rice cultivation, solid waste, coal mining, and natural gas production and distribution. Comparing these emissions to the observed atmospheric increases, the scaling factors for 1987 are that 47 per cent of the CO<sub>2</sub>, 18 per cent of the CH<sub>4</sub> and 100 per cent of the CFCs emitted by any given country represent a net addition to the Earth's atmosphere. To weight each gas for its infrared heating effectiveness compared with CO<sub>2</sub>, we use a factor for CH<sub>4</sub> of 15.8 kg of carbon equivalent per kg of CH<sub>4</sub> (21 to 1 on a per molecule basis); the equivalent factors for CFC-11 and CFC-12 are 1,083 (12,000) and 1,568 (15,800)<sup>1</sup>.

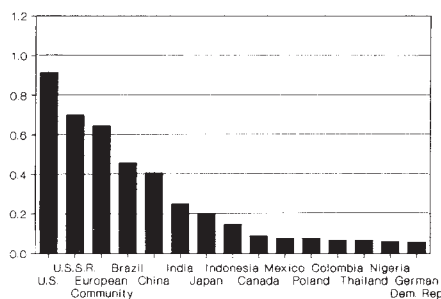
Our greenhouse index provides a more accurate measure of national contributions to potential atmospheric warming than do CO<sub>2</sub> emissions alone. Worldwide, greenhouse gas emissions totalled 5.4 thousand million tons of carbon



equivalents in 1987. The United States and the Soviet Union rank highest on this index, accounting for 17 and 13.1 per cent (see table), respectively, of total global increases in 1987. Overall, industrialized countries, defined as the OECD countries (37 per cent), the Soviet Union (13 per cent) and Eastern Europe (4 per cent), contribute 54 per cent of the total; developing countries account for 46 per cent of the global total.

As emerges from the greenhouse index, however, most climate pollution is concentrated in relatively few countries, both industrial and developed, free-market and planned economies. Six countries that are the largest climate polluters contribute more than 50 per cent of the warming potential: of these, three have heavily industrialized economies and three do not. If the European Community is considered as one unit (see figure), seven of the top fifteen contributors are industrial countries, whereas eight are developing countries. These 15 political entities accounted for 77 per cent of total world additions to atmospheric heating potential in 1987.

In negotiations for a greenhouse convention, developing countries may well assert that equity requires allowance for aspirations of their citizens to improve their lifestyle. For this issue, per capita greenhouse index comparisons are instructive (see table). In 1987, the world average was 1.1 metric tons of carbon equivalent per capita added to the atmosphere annually. National per capita figures varied considerably however. Among industrial countries, the United States had a high per capita contribution, 3.8 metric tons, compared, for example, with Japan's 1.6; EC countries were generally intermediate between these



Top 15 producers for 1987, in CO<sub>2</sub> equivalents, measured by the greenhouse index. Values in thousand million metric tons of carbon.

figures. By comparison China (0.4) and India (0.3) are low. If per capita greenhouse emissions rise markedly in developing countries in response to legitimate aspirations for growth and equity, the impact on rates of greenhouse forcing could be enormous. If, for example, all countries now below the present world average were to increase their per capita emissions to that figure, worldwide annual additions to the atmosphere's greenhouse forcing function would increase by 41 per cent; increasing them to the level of Spain (1.5 metric tons per capita) would increase the worldwide total 69 per cent. Thus the industrialized countries of the world seem to have a powerful incentive both to take the lead in limiting greenhouse gas emissions wherever possible and to assist developing countries in doing the same.

Any global agreement on greenhouse gas emissions will need an accounting system that measures national contributions in a way that is reasonably accurate, simple enough to be interpreted and administered by policy makers rather than scientists, and that can be widely perceived as fair. We believe our greenhouse index can meet these criteria: concentra-

tions of greenhouse gases in the atmosphere are readily measured and accessible to all, unlike the sophisticated models of the carbon cycle. Moreover, this method readily allows trading of integrated 'emission rights' from one country to another, but works equally well with gas-by-gas protocols.

Allowing the atmosphere itself to integrate the effects of biogeochemical cycles is, we believe, preferable to modelling for the purposes of international diplomatic agreements (as opposed to scientific purposes), and also takes into account natural interannual variability. A significant advantage of our method, we believe, is that it avoids the arbitrary element of choosing a time horizon: our experience in developing global datasets of greenhouse gas emissions leads us to believe there will be enough uncertainty and disputes over emission numbers without introducing disputes over time horizons. (In any case, the only logical time horizon, other than one year, would be an infinite one, which is not of interest to most politicians.) Finally, by focusing on instantaneous additions to the atmosphere, our accounting scheme draws attention to current policies and to their immediate effects on the atmosphere and will encourage improved collection of data on emissions. This can increase incentives to make difficult political decisions and, by focusing on results, to reward those countries who do. Brazil, for example, has implemented policies that have substantially reduced its major source of greenhouse gases — CO<sub>2</sub> from deforestation — since 1987, with the result that its greenhouse index based on 1989 data will show a significantly reduced ranking.

Atmospheric scientists have made enormous progress in understanding the interactions between climate pollutants and climate change, but many uncertainties remain. But, as the IPCC report makes clear, there is already a sufficient consensus for governments to begin to reduce their release of climate pollutants and to forge an international agreement to deal with this uniquely global problem. Our analysis illustrates the power of a relatively simple method for implementing this process. □

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THE TOP 20 PRODUCERS AS MEASURED BY THE GREENHOUSE INDEX

| Rank | Country        | Total net additions<br>(per 1,000 metric tons<br>of carbon equivalent) | Percentage share<br>of greenhouse<br>gas increases | Net per capita<br>additions (kilograms<br>of carbon equivalent) |
|------|----------------|------------------------------------------------------------------------|----------------------------------------------------|-----------------------------------------------------------------|
| 1    | United States  | 920,000                                                                | 17.0                                               | 3.8                                                             |
| 2    | USSR           | 700,000                                                                | 13.1                                               | 2.5                                                             |
| 3    | Brazil         | 460,000                                                                | 8.5                                                | 3.3                                                             |
| 4    | China          | 410,000                                                                | 7.6                                                | 0.4                                                             |
| 5    | India          | 250,000                                                                | 4.6                                                | 0.3                                                             |
| 6    | Japan          | 200,000                                                                | 3.7                                                | 1.6                                                             |
| 7    | Indonesia      | 150,000                                                                | 2.7                                                | 0.9                                                             |
| 8    | FRG            | 140,000                                                                | 2.7                                                | 2.4                                                             |
| 9    | United Kingdom | 130,000                                                                | 2.5                                                | 2.3                                                             |
| 10   | Italy          | 100,000                                                                | 1.9                                                | 1.8                                                             |
| 11   | France         | 99,000                                                                 | 1.8                                                | 1.8                                                             |
| 12   | Canada         | 88,000                                                                 | 1.6                                                | 3.4                                                             |
| 13   | Mexico         | 77,000                                                                 | 1.4                                                | 0.9                                                             |
| 14   | Poland         | 76,000                                                                 | 1.4                                                | 2.0                                                             |
| 15   | Colombia       | 67,000                                                                 | 1.2                                                | 2.2                                                             |
| 16   | Thailand       | 67,000                                                                 | 1.2                                                | 1.3                                                             |
| 17   | Nigeria        | 61,000                                                                 | 1.1                                                | 0.6                                                             |
| 18   | Spain          | 59,000                                                                 | 1.1                                                | 1.5                                                             |
| 19   | GDR            | 58,000                                                                 | 1.1                                                | 3.5                                                             |
| 20   | Australia      | 58,000                                                                 | 1.1                                                | 3.6                                                             |
|      | WORLD          | 5,400,000                                                              | 100.0                                              | 1.1                                                             |

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# A tax to avoid the greenhouse?

Enthusiasm for a global tax on carbon used as fuel as a means of forfending global warming is infectiously seductive, but would probably not work and would certainly dodge the main issue in the greenhouse negotiations that lie ahead.

JUST as international relations are too important to be delegated to diplomats, so it is reasonable and welcome that Britain's Royal Institute of International Affairs, otherwise known as Chatham House, should take a close interest in the prospect that the accumulation of greenhouse gases will engender global warming. A few weeks ago, for example, it published an argument by William A. Nitze that the first stab at a greenhouse convention should include restrictions on CO<sub>2</sub> emissions (*The Greenhouse Effect: Formulating a Convention*, Chatham House £10.00), conflicting with this journal's opinion that the most urgent need is to collect as many signatures as possible.

Today, Chatham House publishes the first of two volumes by Michael Grubb under the general title *Energy Policies and the Greenhouse Effect*. The first volume is subtitled *Policy Appraisal* (Dartmouth Publishing, £25.00 plus £2.50 postage and packing). The second, including case studies of the energy economies of several different countries, is due early next year. On the showing of the first volume, the enterprise promises an invaluable collection of data and argument. The underlying assumption is that there will be an international convention, and soon. Among other things, Grubb emphasises the importance of chlorofluorocarbons in the present pattern of greenhouse forcing — 24 per cent, compared with 55 per cent for CO<sub>2</sub> — suggesting that this is an area in which to act soon.

But then Grubb concentrates on the energy business, on the grounds that much of the rapidly growing burden of methane in the atmosphere, responsible for 15 per cent of present greenhouse forcing, derives from the extraction of coal and leakage from the oil and gas industries' installations. And he advocates a carbon tax, preferably uniform throughout the world, to discourage the relocation of industry to where carbon taxes are least. This would be his chief weapon in the reduction of greenhouse emissions, although there are careful discussions of schemes for providing people with incentives to use energy efficiently as well as of research renewable energy resources. Except on the carbon tax, Grubb is not prescriptive; his book will be all the more valuable on that account.

The seductiveness of a global carbon tax is easily understood, of course. It is a market solution, providing a single objective regulator by means of which the

world's emission of CO<sub>2</sub> could be controlled. One imagines a committee of the World Meteorological Organization and the UN Environmental Programme meeting towards the end of every year to fix the tax for the succeeding year, which each signatory would then be obliged to levy. Better still, as Grubb argues, fixing the tax rate for a decade ahead (at, say, 4 per cent above the inflation rate) could let the world's industrialists know where they stood, thus minimizing the economic disruption likely to be caused by too rapid an introduction of a carbon tax.

But the notion is unlikely to capture signatures of an international convention. The chief obstacle is the prickliness of governments about national sovereignty. It is just conceivable that a substantial proportion of those attending next February's meeting in Washington (to begin the negotiation of a greenhouse convention) will accept an obligation that CO<sub>2</sub> emissions may have to be constrained, but hardly so that they will agree to exercise restraint by means of an externally determined tax internally administered.

Governments willing to accept restraining obligations will more probably insist that they alone should decide how best to satisfy them. Needless to say, there are also practical difficulties of collection as well as those of telling what the rate should be in fuel-rich centrally planned economies whose currencies are not convertible — China, for example, and the Soviet Union for the time being.

There is a stronger case for a carbon tax at the national level. One of the first results would be that coal, with no hydrogen worth speaking of, would be penalized relative to oil and especially to natural gas. But what would be the effects on the economy at large? Grubb's review of what economists have to say on the issue seems comprehensive, which goes to create the impression that there is much still to be understood.

Of course, a carbon tax would be a market incentive to the efficient use of fuel, but it seems that it is still not clear to what degree the oil-shocks of 1973 and 1979, which were both inflationary (by increasing prices) and deflationary (by diverting cash from other purchases and from investment), contributed to the recessions that followed them. Grubb's case is that a national carbon tax would not be the equivalent to an externally determined price increase because the revenue it would raise could be used

to offset other taxes.

Grubb quotes the several studies that have even shown that a carbon tax might stimulate a national economy. How could it be that increasing the price of an essential commodity could have such an effect? One mechanism is that, in the hunt for fuel efficiency, people would invest in more efficient appliances, not to mention heat insulation and double glazing, all of them requiring people working in manufacturing industry. A more subtle mechanism is the means by which reduced taxes would leave many people with more to spend on commodities other than energy, or for investment, so that the gross domestic product might actually increase as the carbon tax is raised. These are tantalizing notions, which deserve more careful working through.

Where Grubb is surely on safe ground is in his protest at the continuing practice of governments of subsidizing energy, where the United States is the worst offender. (On one estimate, US energy subsidies in the form of tax rebates for oil drillers and mine operators, not to mention the activities of the US Corps of Engineers, amount to \$40,000 million a year.) The economists' goal of internalizing (or making explicit) the externalities (the hidden adventitious costs, that of greenhouse warming in particular) is admirable (as in the principle of "making the polluter pay"); in the context of a greenhouse convention, energy subsidies work in the other direction, and are thoroughly reprehensible. But will not the abolition of subsidies damage especially the poor and the elderly, who may not then be able to get to work or to keep warm? Grubb argues that the only rational solution is to make cash payments to those who need them. He is correct, but the case is not popular with governments.

The chief value of the economic section of this volume is that it shows how much more the economists must do. But the most serious deficiency in the greenhouse argument remains that of partitioning allowable emissions equitably between industrialized and developing countries. The eagerness of prosperous countries (Britain, for example) to offer not to increase emissions above those of some nominated previous year if others will follow suit is incompatible with avoiding global warming and allowing the development of the poor countries. And that is the nub of the argument yet to come.

John Maddox

# A case of vanishing neutrinos

Michael Cherry

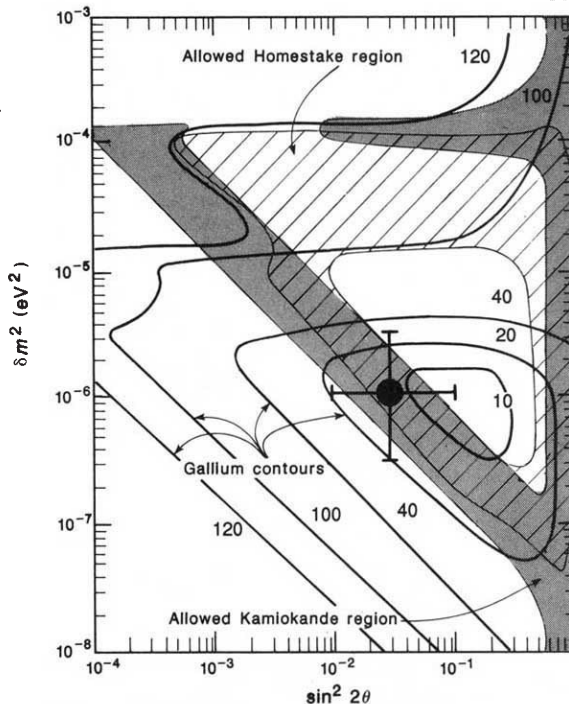
DIRECT measurements of the Sun's interior can be obtained either from seismic observations of solar oscillations or from studies of the neutrinos produced by nuclear fusion reactions in the Sun's core. For about two decades now, the radiochemical chlorine-37 solar neutrino experiment in the Homestake Gold Mine<sup>1</sup> has been monitoring the flux of relatively high-energy neutrinos (mainly from boron-8 decays) with the count rate consistently a factor of 3–4 below the value expected from standard models of solar physics<sup>2,3</sup>. Apparently, either our models of the Sun's interior are incomplete or our understanding of neutrino physics is deficient<sup>4</sup>. Although numerous non-standard models of solar physics have been put forward to account for this diminished neutrino flux, none has met with general approval. Several new results, moreover, seem to be pointing to problems with the neutrinos.

New support for the standard solar model came from the helioseismological study by Elsworth *et al.*<sup>5</sup> of low-degree pressure modes (p-modes), based on solar velocity measurements at the Sun's surface. The frequency differences between low-degree modes are believed to be relatively independent of effects due to the solar surface and so provide some sensitivity to the interior structure. Using data collected over the past 8 years at several detection sites, Elsworth *et al.* find that the p-mode fine structure agrees better with predictions from a number of standard models than with those from a selection of non-standard models: their conclusion is that the solar-neutrino deficiency must result from neutrino physics, not solar physics.

The most likely explanation involving neutrino physics is based on Mikheyev-Smirnov-Wolfenstein (MSW) neutrino oscillations in the solar interior<sup>6,7</sup>. These oscillations change the character, or flavour, of the neutrinos from electron-type which can be detected in the current experiments, to muon- or tau-type, which cannot. The degree to which these flavours interchange, or mix, depends on the neutrino mass differences and the 'mixing angles'. Assuming that the Sun's core actually produces the expected number of electron neutrinos, the suppression required to reproduce the Homestake result can be obtained in three ways: with a large mass difference ( $\delta m^2 \sim 10^{-4} \text{ eV}^2$ ); with a large mixing angle ( $\sin^2 2\theta \sim 1$ ); or with  $\delta m^2 \times \sin^2 2\theta \sim 3 \times 10^{-8} \text{ eV}^2$ .

In the first, high-mass-difference case,

the oscillations occur preferentially at higher energies, so that a measurement of the neutrino energy spectrum should show a suppression at the high-energy end. The group at the Kamiokande II neutrino detector in Japan has just presented data<sup>8</sup> from 1,040 days of observation that con-



Neutrino oscillations: the regions in mass-difference–mixing-angle space allowed by experimental results. The shaded region is the area permitted (with 95 per cent confidence) by Kamiokande II; the cross-hatched area, that allowed by Homestake. Both regions contract and the intersection region decreases at the 68 per cent confidence level. The point gives Bahcall and Bethe's prediction. The solid lines correspond to gallium counting rates of 10–120 SNU.

firm the total deficit observed at Homestake and which fit the unsuppressed <sup>8</sup>B spectrum. By detecting electrons recoiling from collisions with high-energy neutrinos, the Kamiokande II detector can both verify that the neutrinos come from the Sun (unlike the Homestake detector which yields no directional information) and measure the energy in individual events.

The Kamiokande group records a neutrino detection rate  $0.46 \pm 0.05$  (statistical)  $\pm 0.06$  (systematic) times that predicted by Bahcall and Ulrich's standard solar model<sup>2</sup>. (There is no sign of the large-timescale variations reported by Homestake, which have been related to the solar cycle.) The energy spectrum for neutrinos in excess of 7.5 MeV is in excellent agreement with that expected for <sup>8</sup>B decays: there is no evidence of suppression of electron neutrinos at high energy, weakening the case for the high-mass-difference MSW mechanism for all

but the largest values of  $\sin^2 2\theta$ .

In the large-mixing-angle case for the neutrino mixing, the oscillations are essentially energy independent. Bahcall and Bethe<sup>9</sup> argue that according to the Bahcall–Ulrich standard model with an energy-independent suppression factor derived from the Kamiokande II result, the <sup>8</sup>B detection rate recorded at the Homestake experiment should be 2.8 solar neutrino units (SNU). If one then includes also the neutrino flux expected from <sup>7</sup>Be and from proton–electron–proton fusion reactions, the count rate at Homestake should rise to 4 SNU, well above the  $2.1 \pm 0.3$  SNU actually measured. Bahcall and Bethe therefore discount the possibility of energy-independent oscillations.

For the third solution, one again requires consistency between the Homestake and Kamiokande results: using the Bahcall–Ulrich standard model and MSW oscillations, Bahcall and Bethe derive a neutrino mass difference  $\delta m^2 \sim 10^{-6} \text{ eV}^2$  and  $\sin^2 2\theta \sim 0.03$ . The neutrino suppression would then occur mainly at low energy, below the minimum energies observable at the Homestake and Kamiokande II detectors.

The only detectors currently capable of observing the low end of the solar neutrino spectrum are based on gallium. Of the total 132 SNU predicted to be seen in gallium detectors (in the absence of neutrino oscillations), 70 come from the proton–proton (p–p) fusion reaction that is the fundamental source of power from the Sun. Bahcall and Bethe's suggested parameters would reduce this rate to 5 SNU. The preliminary data<sup>10</sup> from the Soviet–American Gallium Experiment (SAGE) indicate a best fit of 0 SNU and an upper limit (95 per cent confidence) of 135 SNU. At 68 per cent confidence, the upper limit is 72 SNU.

Whereas the high-energy detection rate depends sensitively on details of the solar physics (the <sup>8</sup>B rate depends on the eighteenth power of the core temperature), the 70 SNU p–p rate expected in gallium is a direct result of the assumption that the Sun burns primarily through the

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reaction chain  $p + p \rightarrow {}^3\text{H} + e^+ + \nu_e$ ,  ${}^2\text{H} + p \rightarrow {}^3\text{He} + \gamma$ ,  ${}^3\text{He} + {}^3\text{He} \rightarrow {}^4\text{He} + 2p$ . An observed gallium rate of less than 70 SNU therefore cannot be explained by changing the solar models. The statistics from SAGE are still poor, but if this low result is sustained, it may be a real indication of new neutrino physics. SAGE is currently doubling the size of its gallium metal target from 30 to 60 tons and accumulating

additional data. In addition, the results from a second gallium detector (GALLEX, located in the Italian Gran Sasso Laboratory) are due soon. Can we expect a shake up in particle physics to follow shortly? □

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## DRUG METABOLISM

# Growth signal pathways

*Daniel W. Nebert*

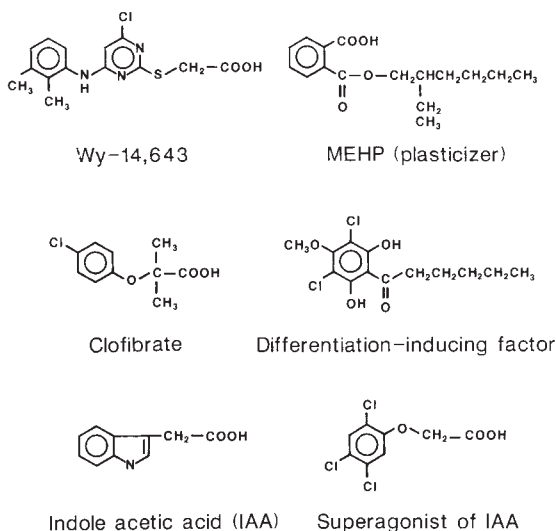
A STEP towards the development of a theory linking receptor-mediated growth transduction pathways, and the mechanisms by which foreign chemicals as well as endogenous substances act as ligands, is made with the report of Issemann and Green that appeared in *Nature*<sup>1</sup> last week. The achievement is in the cloning and characterization of the mouse peroxisome proliferator activated receptor (mPPAR), a novel member of the nuclear receptor superfamily, and in the demonstration that the only ligands for it appear to be foreign chemicals.

The nuclear receptor superfamily includes the glucocorticoid, mineralocorticoid, oestrogen, progesterone, androgen, ecdysone, retinoic acid, vitamin D and thyroid hormone receptors. The genes for all of these receptors have been cloned. All of the receptors bind with high affinity to their endogenous ligands and have a critical role in a variety of neuroendocrine and other signal transduction pathways leading to growth, morphogenesis, homeostasis and proliferation<sup>2</sup>. Also expected to be in the nuclear receptor superfamily, and for which the only known ligands are foreign chemicals, are the dioxin receptor (aromatic hydrocarbon receptor) and the phenobarbital receptor.

After confirming that they had isolated the mPPAR gene, Issemann and Green went on to demonstrate the receptor's function. They used chimaeric expression plasmids in which the complementary DNA encoding the probable ligand-binding domain of the receptor is ligated downstream of the cDNA encoding the DNA-binding domains of either the oestrogen receptor gene or glucocorticoid receptor gene. The transfected chimaeric plasmid expresses a chimaeric receptor protein which, when activated by the proper ligand, can bind to a second transfected plasmid containing either the oestrogen response element or the glucocorticoid response element, either of which drives the chloramphenicol acetyltransferase (CAT) reporter gene.

For both systems, Issemann and Green found that increases in CAT activity are

caused by a class of chemicals that stimulate the proliferation of hepatic peroxisomes, but not by many other ligands. Also, the level of CAT activity correlated well with how strongly the ligand induces cancer as well as peroxisomal proliferation. This elegant approach will undoubtedly



A comparison of the structures of foreign chemicals (Wy-14,643, mono-ethylhexyl phthalate (MEHP) and clofibrate) and endogenous ligands (IAA and its superagonist, and differentiation-inducing factor).

edly be useful for identifying the ligands of other novel nuclear hormone receptors.

One reason for thinking that Green and Issemann's results are of considerable significance is that the rank order of chemicals they show to be the most potent peroxisome proliferators is the same as the rank order of chemicals that are most potent in receptor-mediated activation of genes encoding P450 and other drug-metabolizing enzymes. The explanation, I propose, is that drug-metabolizing enzymes are responsible for controlling the steady-state levels of small bio-organic oxygenated molecules (including steroids) that act as signals for growth, differentiation, virulence and even tumour promotion, and that the mPPAR mediates one of many such growth transduction pathways.

Why should an animal have receptors with high affinity for foreign chemicals such as peroxisome proliferators, dioxin or phenobarbital? Clearly, the organism has not been waiting hundreds of millions of years to be challenged by drugs or environmental pollutants. I suggest that these foreign chemicals are structurally similar enough to mimic an endogenous ligand that has an important role in growth or morphogenesis. The ligands could be bio-organic oxygenated molecules not that different in size or shape from the steroids, retinoic acid or other ligands described above. The figure shows subtle similarities in the chemical structures of Wy-14,643, the plasticizer MEHP, and clofibrate — among the most potent ligands of the mPPAR (ref. 1). These structures have features in common with those of the plant auxin (growth hormone) indole acetic acid, the active ingredient in the herbicide Agent Orange (2,4,5-trichlorophenoxyacetic acid), which is a potent agonist of indole acetic acid, and the differentiation-inducing factor in the slime mould *Dictyostelium discoideum*<sup>3</sup>.

Foreign chemicals often act as agonists or antagonists of endogenous ligands. For example, the insecticides 1,1,1-trichloro-2,2-bis-(*p*-chlorophenyl)-ethane (DDT) and kepone can bind to the oestrogen receptor and exert an oestrogenic response<sup>4</sup>. Dioxin causes birth defects in amphibians as well as mammals<sup>5</sup>. The teratogenic activity of dioxin requires the presence of the aromatic hydrocarbon receptor, suggesting that the binding of dioxin interferes with an important role of this receptor in differentiation and growth. Interestingly, peroxisome proliferators, dioxin and phenobarbital all cause cell proliferation and are all tissue-specific tumour promoters<sup>6</sup>.

What, then, is the relationship between the compounds shown in the figure and probably all the other chemicals discussed here? Small bio-organic growth effector molecules are synthesized at least in part by P450-mediated reactions; they are also metabolically degraded by P450 and other drug-metabolizing enzymes. Further, all of these ligands activate genes encoding particular forms of cytochrome P450 and other drug-metabolizing enzymes.

Many classes of substances, or types of stimuli, are known to activate P450 gene expression<sup>7-9</sup>. They have several features in common. First, all are involved in growth — the up- or down-regulation of cell cycle-specific genes, including differentiation, in eukaryotes, the metabolism of energy substrates, and cell division-specific and replication-specific genes in



bacteria. That phenobarbital activates P450 genes in animals, plants and bacteria implies that it mimics a universal regulator of growth. Second, many of the effectors cause proliferation or tumour promotion in a particular eukaryotic tissue or cell type<sup>9</sup>; cell proliferation is caused by chemicals that at high doses induce cancer<sup>10</sup>. Third, many of the chemicals that activate particular P450 genes are ligands for receptors in the nuclear receptor gene superfamily.

Therefore, the activation of genes encoding P450 as well as other drug-metabolizing enzymes by the growth effectors might be an important step, upstream in the regulatory cascade, for controlling the steady-state levels of the various transcription-activating factors. Alternatively (or additionally) the function of P450 genes might involve the metabolism of other second messengers (for example, arachidonic acid metabolites, prostaglandins, leukotrienes) in the regulatory cascade controlling cell cycle-specific genes.

The discovery of the mPPAR gene<sup>1</sup> brings us closer to understanding the intricate interrelationships between a

huge variety of biological phenomena, ranging from bacterial growth and cell division to ischaemic heart disease. All of these processes are related to growth and differentiation, in which members of the nuclear hormone receptor superfamily and genes encoding drug-metabolizing enzymes share centre stage. It remains to be seen, however, whether the steady-state levels of most important bio-organic oxygenated growth-effector molecules are controlled by the drug-metabolizing enzymes. □

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## PLANT ECOLOGY

# Vegetation's place in history

Peter D. Moore

FOR the past 70 years there have been two strongly opposed camps among students of vegetation. The members of one group, inspired by the ideas of Clements<sup>1</sup>, view vegetation as a set of units, associations or communities, in which the various component species have co-evolved in a manner that ensures an optimization of resource exploitation and a minimum of niche overlap. Clements regarded such integrated structures as organic entities, similar to living organisms. The alternative view, pioneered by Gleason<sup>2</sup>, perceives vegetation as an assemblage of individual plants belonging to a variety of species, with each species distributed according to its own physiological requirements, as constrained by competitive interactions. The hypotheses have been vigorously defended by descriptive accounts of current vegetation, with various studies showing either that species tend to cluster in groups, or that they are spread as a continuum. But research into the history of vegetation, as manifested in three recent reports<sup>3–5</sup>, may hold the ultimate key to the controversy, for it shows that post-glacial movements of species have not occurred in concert and that the timescale involved may have been too short for extensive co-evolution.

The techniques available for examining the history of a plant community depend upon timescale. When considering

decades or even centuries, it may be possible to use documentary sources, but longer timescales require the use of fossil reconstruction, often involving the use of pollen stratigraphy in peats and lake sediments. In the case of the beech-hemlock (*Fagus grandifolia*–*Tsuga canadensis*) forests of the northeastern United States, both techniques have been applied. These two trees are currently important members of the mixed conifer-hardwood forests of the Great Lakes and New England area and their present geographical ranges are similar. They certainly seem to be strong contenders for members of an established community.

Whitney<sup>3</sup> has traced the history of the beech-hemlock forest of the Allegheny plateau of Pennsylvania back for 200 years using forest surveys from the early nineteenth century. At that time beech comprised about 43 per cent of the tree composition of the forest and hemlock about 20 per cent. Since then the exploitation of the area's forest resources has resulted in considerable changes favouring red maple (*Acer rubrum*), black cherry (*Prunus serotina*) and sugar maple (*A. saccharum*). But the antiquity of the beech-hemlock forest has been established and the woodland assemblage evidently pre-dates the arrival of European settlers in the area.

Analyses over longer timescales,

involving the mapping of pollen abundance through the past 12,000 years, were carried out a few years ago by Jacobson, Webb and Grimm<sup>6</sup>, and have now been reconsidered by Graham and Grimm<sup>4</sup>. Reconstructions of the main centres of distribution of hemlock and beech (defined as areas in which the pollen representation of these types equalled or exceeded 5 per cent of the total terrestrial pollen) show that their ranges have overlapped strongly for only about 6,000 years, and have been fully coincident for only perhaps 500 years. At the end of the Wisconsin glaciation, some 12,000 years ago, the two taxa occupied very different regions of the southeastern corner of North America, with hemlock in the elevated regions of the southern Appalachian mountain chain and beech occupying the lowland regions. Both moved gradually north, but they became important members of the same woodland 'community' only in the mid-Holocene. As Graham and Grimm point out, the response of these plant species to climatic change is essentially individualistic. Their association in the Great Lakes forests is a geologically very recent event.

The organismal concept of community is essentially dependent on the co-evolution of the component species, and the important question is whether there has been enough time to permit such mutual genetic adjustments during the limited time span of the Holocene (10,000 years). Earth's climate has been unstable for the past two million years, most of that period being characterized by glaciations. Bennett<sup>5</sup> proposes that the time available for evolutionary development to cope with the warm interruptions (interglacials) has been too short for most species, particularly those, such as trees, with relatively long generation times. No sooner is an assemblage of species gathered than the next cold event commences.

Species, it would seem in these unstable days, are wandering the Earth in response to their physiological limitations and their competitive capacities, complicated in some cases by sheer historical accident. Adding a time dimension to the debate about vegetation patterns makes it all the more difficult to accept the concept of organismal units that can be identified, named, classified and even sought in the fossil record. □

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# Growing from below

Roberta Rudnick

THE lower crust of the continents, some 20–40 km deep in the Earth, is inaccessible to Earth scientists. The deepest continental drilling has penetrated only 12 km, so that the only known samples of the lower crust are high-pressure rocks in granulite facies brought to the surface either as outcrops owing to tectonic processes or as xenoliths in certain volcanoes. Voshage *et al.*, on page 731 of this issue<sup>1</sup>, report new analyses of surface granulites from the remarkable Ivrea zone, an outcrop in the Italian Alps representing an 8.5-km cross-section through the lower crust<sup>2</sup>, and show from their composition that the lower crust grew through underplating by magma derived from the Earth's mantle.

The rocks of the Ivrea zone are unusual when compared with other granulite outcrops because they contain a large proportion of mafic rocks and, as such, closely resemble granulite xenoliths in composition. The section examined by Voshage *et al.* comprises mafic granulites, which were formed by the intrusion into existing crust and subsequent fractionation of basaltic melts that partially melted the surrounding metasedimentary rocks. The authors show that although the isotope compositions of the cumulates are highly variable in the lower 200 metres, the magma evolved to a remarkably homogeneous isotope composition which is maintained through the remaining upper 8 km of the section. In contrast to this isotopic uniformity, the concentrations of incompatible trace elements increase progressively up through the section.

Voshage *et al.*<sup>1</sup> attribute these features to crustal growth via several acronymic processes arising in the magma chamber formed by the intruding melts: AFC (assimilation and fractional crystallization<sup>3</sup>), MASH (melting, assimilation, storage and homogenization<sup>4</sup>) and RTF (refilled, tapped and fractionated<sup>5</sup>). It is the MASH and RTF processes that are believed to be responsible for the isotopically homogeneous but chemically variable nature of the main body of the intrusion. In the authors' model, the amount of crust assimilated is proportional to the heat added through replenishment of the magma chamber by hot, mantle-derived magmas. Thus the isotope composition of the hybrid magma, which reflects this ratio, remains constant, but the incompatible trace-element concentrations increase as the intrusion crystallizes. The similarity in isotope composition and age between the Ivrea zone cumulates and granites in the upper crust dating from the Hercynian mountain-building episode allow Voshage *et al.* to speculate that

these granites have been derived from lower-crustal magma chambers similar to those in the Ivrea zone. In addition, the pervasive positive europium anomaly present through most of the Ivrea granu-



MASHed rocks. Injection of basaltic magma into a deep-crustal magma chamber causes partial melting of the metasedimentary rocks that form the roof of the chamber. In the outcrop shown here, the melting (white bands) has been so extensive that it is easy to imagine this process leading to disaggregation of the crust and its assimilation into the basaltic magma. (Photograph courtesy of A. W. Hofmann.)

lite sequence leads to the conclusion that crystal cumulates such as these may represent the lower-crustal reservoir complementary to the upper-crustal negative anomaly<sup>6</sup>.

Conclusions similar to those reached by Voshage *et al.* regarding the role of basaltic underplating in formation and modification of the lower continental crust have been drawn previously from the widespread occurrence of mafic lower-crustal xenoliths<sup>7</sup>. The data of Voshage *et al.*, however, allow one to estimate the amount of new crust formed by this process, a task that is difficult, if not impossible, to perform using xenolith data. Using an average mixture of 1 part pre-existing lower crust to 2 parts intruded basaltic magma for the Ivrea zone intrusion, as assumed by Voshage *et al.*, one can estimate that approximately 6 km of crust

(roughly 17 per cent of a 35-km-thick crust) was formed by the underplating of basaltic magma in the late Palaeozoic. This is a minimum estimate, taking into account only material present in the exposed intrusion. If significant amounts of melt were extracted from this intrusion, then the amount of new crustal addition would be even greater. This estimate of new crust formed by basaltic underplating

is in harmony with calculations of the amount of melt that can be generated and can underplate the lower crust through mantle upwelling<sup>8</sup>.

Another important aspect of the new study is the demonstration that large volumes of hybrid magmas of homogeneous composition form in the lower crust by mixing between crust and mantle-derived basalts. This finding, suggested previously by Hildreth and Moorbath<sup>4</sup> from studies of eruptive rocks in the Andes (see the previous News and Views article<sup>9</sup> by Marjorie Wilson), confirms the importance of deep crustal magma chambers in homogenizing isotope compositions of derivative magmas.

The data from the Ivrea zone, along with those from lower-crustal xenoliths, demonstrate that basaltic underplating is an important means of crustal growth. This type of process may occur above subduction zones, in continental rift zones or intraplate settings — anywhere that mantle upwelling is likely to produce basaltic magmas. Thus, although lateral accretion of continental crust through addition of island arcs remains an important crustal growth process, it must not be considered the only means by which continents grow.

Finally, if basaltic underplating is an

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important crustal growth process, then we are faced with a paradox: the bulk composition of the crust is not basaltic<sup>6</sup>, yet only basaltic magmas are derived from the mantle. This is one of the great unsolved dilemmas of crustal geochemistry. Potential solutions to this problem include derivation of non-basaltic magmas from the mantle during the Archaean owing

to higher geothermal gradients<sup>10,11</sup> or delamination of mafic and/or ultramafic cumulates from the base of the continental crust<sup>12,13</sup>. Both hypotheses remain speculative; both deserve further evaluation and testing. □

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## LEUKAEMIA AND NUCLEAR FACILITIES

# Data in search of explanation

H. John Evans

In this issue of *Nature* (page 755), Catherine Hill and Agnès Laplanche<sup>1</sup> report on their study of deaths from childhood cancer in the vicinity of six French nuclear installations (including the reprocessing plants at La Hague and Marcoule). Although workers in Britain have shown there is an excess of childhood leukaemias near certain nuclear establishments<sup>2,3</sup>, the French workers find none.

Further fuel to the debate is added by two other recent reports — one from the United States on cancer mortality in counties containing nuclear installations, which was discussed in a news item in these pages<sup>4</sup>; the other by Leo Kinlen and colleagues<sup>5</sup>, which adds support to Kinlen's hypothesis that some childhood leukaemias are the result of an unidentified infection that is promoted by the mixing together of a large number of people of diverse origins.

The French study was based upon cancer mortality, between 1968 and 1987, in the 0–24-year age group within each of four concentric zones extending up to 16 km from each of the nuclear plants. The electoral units ('communes') within each zone were identified; and a control commune was selected, from within the same administrative 'département', that had an equivalent total population and that is, on the average, some 53 km from the relevant installation.

## Comparisons

The information on mortality was obtained from death certificates, while national census returns provided population data on the communes. Two comparisons were made — the observed mortality ascribed to neoplastic disease against overall national rates, and (to minimize possible systematic differences in national certification procedures) mortality ascribed to neoplastic disease against mortality in the selected control communes. Two of the principal findings are, first, that there were no differences in the overall cancer deaths in the groups that were compared; and second, that the number of leukaemia deaths recorded in the population residing in the vicinity of the nuclear installations (58) was similar

to that recorded in the control areas (62) and to that expected from national mortality statistics (67). This latter finding confirms, and very much extends, an earlier preliminary report<sup>6</sup> on leukaemia mortality around the nuclear reprocessing plant at La Hague.

A similar failure to demonstrate increases in cancer mortality (including childhood leukaemia) comes from the survey in the United States. The survey, which was recently announced by the US National Cancer Institute (NCI), covered counties containing nuclear installations before and after the commissioning of these plants (>900,000 cancer deaths) and compared them with counties without nuclear facilities (>1,800,000 cancer deaths). The authors of the report acknowledge that the sizes of the 107 counties analysed may have been too large to detect higher incidences of cancer that might have been confined to the close vicinity of nuclear plants; but one should note that the same type of county analysis had previously shown an increased risk of lung cancer associated with exposure to arsenic released by smelters into the local environment.

The French and US findings might appear, at least in part, to conflict with those that emerged from the Office of Population Censuses and Survey data on British nuclear installations analysed by Forman *et al.*<sup>3</sup>. The British data also revealed no general increase in cancer mortality near nuclear installations, but they did provide evidence for an increased frequency of deaths from leukaemia, specifically lymphoid leukaemia, in the 0–24-year age group. It was, however, noted that the significant excess of lymphoid leukaemia in the local authority areas close to nuclear installations depended in large part on the unusually low occurrence of childhood lymphoid (but not non-lymphoid) leukaemia deaths in the comparable control regions.

Enter Leo Kinlen, and his colleagues, who have now published their study of deaths from childhood leukaemia in 14 'new towns' in Britain<sup>5</sup>. Nine of the towns are 'overspill' new towns that have received an already well-mixed popula-

tion from a nearby city (London or Glasgow, for example). The remaining five are 'rural' new towns (such as Aycliffe or Glenrothes) which were set up to house the populations serving several industrial development areas; it is known from migration details that these populations have a wider variety of origins than those in the overspill groups. In the decade following their establishment, the density of children in both types of town rose rapidly, but the density of preschool children in the rural new towns was very much greater than that in their principal cities of origin or in the overspill new towns. Comparisons of observed to expected deaths from leukaemia showed a significant excess in early years after the establishment of the new towns, which were evident in the rural but not in the overspill new towns. The excesses were largely due to deaths from lymphocytic leukaemia in the preschool (0–4-year) age group (as at Sellafield<sup>2</sup>); were greatest where the population density was highest; and were associated with significant deficits of leukaemia deaths in the 5–24-year age group.

## Unrecognized infection

Kinlen *et al.* reject certain interpretations of some of their findings (such as differences in the success rates for the treatment of childhood leukaemias in the different towns, or that the effects are due to mutations resulting from a delayed exposure to infection). Instead, they argue that the patterns they observe are precisely those that would be expected if an unrecognized infection, similar to feline leukaemia virus infection and occurring *in utero* or in children of preschool ages, was responsible for the excesses of childhood leukaemia in the early age group and for the deficit, as a consequence of the immunizing effects of the infection, in the later age group.

The findings in France and the United States provide some reassurance on the absence of any general increase in cancer, including childhood leukaemias, near nuclear installations. In contrast, a study in the United States, carried out by the Massachusetts Department of Public Health, is reported<sup>6</sup> to have found an increased incidence of adult leukaemias in people who lived and worked within ten miles of the Pilgrim nuclear power plant in Massachusetts — apparently described by federal officials as "the worst run" nuclear plant in the United States — as compared with incidences in more distant communities. The report claims to show a four-fold increase in adult leukaemias in the years 1978–83, but no excess in the period 1983–86. The Pilgrim study, although smaller than the NCI study, was focused on a small area surrounding a single nuclear plant and analysed incidences of leukaemia rather than mortality.

Attempts were made to minimize confounding factors, but in the absence of details of the methods used in the study it is not possible to comment on the strength of the reported finding.

None of the studies in France and the United States yield information of a sort that would help to understand the nature and causes of the localized excesses of childhood leukaemias apparent in some British studies, in particular those associated with Sellafield. The detailed case-control studies at Sellafield have revealed an association between paternal occupation and childhood leukaemia risk in offspring, and film-badge dose recordings have been interpreted as indicating a pre-conceptual effect of paternal exposure to radiation. The levels of exposure to external radiations in these cases were low, however, and are considered by most geneticists to be insufficient to result in the

postulated induction of predisposing mutations in parental germ cells (see my previous News and Views article<sup>9</sup>). On the other hand, population mixing and an aetiology associated with infection is clearly becoming a more attractive proposition to account for these small but significant and tragic excesses. □

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## CELL BIOLOGY

# A new direction for kinesin

Fady Malik and Ron Vale

MICROTUBULE-based protein motors power the movements of chromosomes in mitosis, the transport of membranous organelles, and the beating of cilia and flagella. To understand the function of a given motor, it is important to determine its direction of movement along the microtubule, which is a polarized filament with distinct 'plus' and 'minus' ends. Recently, Endow *et al.*<sup>1</sup> and McDonald and Goldstein<sup>2</sup> demonstrated that the predicted amino-acid sequence of the protein encoded by the *Drosophila melanogaster claret nondisjunctional* gene (termed *ca<sup>nd</sup>* or *ncd*) contains a domain that is 40–45 per cent identical to the motor domain of kinesin, a plus-end-directed motor. On the basis of this similarity, it was expected that the *ncd* gene product, which is involved in chromosome segregation during female meiosis, would turn out to function as a plus-end-directed motor. Walker *et al.*<sup>3</sup> (page 780 of this issue), and, independently, H. McDonald, R. Stewart and L. Goldstein (personal communication), have found that the *ncd* protein expressed in *Escherichia coli* is indeed a microtubule motor when assayed *in vitro*. Surprisingly, however, it moves towards the microtubule's minus-end, a direction previously thought to be driven by dynein but not by kinesin-like motors.

Until now, it has seemed that two structurally and enzymatically distinct types of motors (kinesin and dynein) evolved to handle the chores of minus- and plus-end-directed microtubule movements<sup>4</sup>. There is a remarkable difference in size between their ATP-binding subunits (kinesin *M*<sub>1</sub> 100–140K, dynein *M*<sub>1</sub> 400–500K). Furthermore, both motors differ greatly in their

ability to hydrolyse different nucleotide substrates and in their sensitivity to ATPase inhibitors.

In the past year, genetic studies have uncovered five genes that contain domains bearing significant similarity (30–45 per cent amino-acid identity) to the *Drosophila* kinesin mechanochemical domain. The products of the *nod* (ref. 5) and *ncd* (refs 1, 2) genes of *Drosophila*, the *cut7* (ref. 6) gene of *Schizosaccharomyces pombe*, and the *bimC* (ref. 7) gene of *Aspergillus nidulans* are required during mitosis or meiosis, whereas the *KAR3* (ref. 8) gene product from *Saccharomyces cerevisiae* is required for nuclear migration along microtubules during karyogamy. The location of the 350-amino-acid mechanochemical domain of these putative motors is either at the amino terminus (*nod*, *cut7*, *bimC*) like conventional kinesin, or at the very carboxy terminus (*ncd*, *KAR3*). There is no sequence similarity outside the mechanochemical domain for any of these genes. Collectively, these findings suggest the existence of a superfamily of kinesin motors whose members contain a similar force-generating head connected to different tails that perhaps allow them to attach to and move different objects in the cell. The supposition, based on their similarity to conventional kinesin, is that members of the kinesin superfamily all function as plus-end microtubule motors. There has been some lingering suspicion of this idea, though, because the role of the *KAR3* protein in karyogamy can be better explained if it is a minus-end-directed motor.

Motility assays *in vitro* enable the dir-

ection of movement and other motile properties of purified motors to be determined. The low abundance of many of the kinesin-related motors makes their biochemical purification a difficult task. Another route was paved by the work of Yang *et al.*<sup>9</sup>, who expressed kinesin or portions of kinesin in *E. coli* and used the motility assay to define the mechanochemical domain.

Walker *et al.*<sup>3</sup> and McDonald *et al.* used a similar strategy to show that bacterially expressed *ncd* protein moves microtubules *in vitro*, but towards the microtubule minus end, the direction opposite to kinesin-driven movement. Also the sensitivity of *ncd* to inhibitors of motility was more similar to dynein than kinesin. A further surprising finding was that *ncd* rotates the microtubule about its longitudinal axis while translocating it forward. Such torque generation has previously been observed only with the 14S dynein from *Tetrahymena* cilia and never with kinesin.

Even though only minus-end-directed motility was detected with *ncd*, Walker *et al.* mention that *ncd* could produce plus-end-directed movement, perhaps under appropriate regulation. This idea would seem heretical were it not for the work of Schliwa and colleagues<sup>10</sup> on a dynein-like (*M*<sub>1</sub> 400–500K subunit) organelle transport motor from the freshwater amoeba, *Reticulomyxa*. This motor induces bidirectional movement of beads along microtubules<sup>11</sup>; but it was difficult to rule out the possibility that a kinesin-like contaminant is responsible for the plus-end-directed motility. Their recent study, however, puts the conclusion that the *Reticulomyxa* dynein is a bidirectional motor on firmer ground.

Schliwa *et al.*<sup>10</sup> examined 15 nucleotides and nucleotide analogues to see which would support plus- and minus-end-directed movements of organelles in a detergent-permeabilized cell model of *Reticulomyxa*. The nucleotide 'fingerprint' of both plus- and minus-end-directed organelle transport was more characteristic of dynein than of kinesin. In addition, they found that the velocities of organelle transport in both directions were indistinguishable using this panel of substrates. The finding is surprising because all motors tested so far, including several dynein motors, can be distinguished by their substrate specificities. The results imply that the same dynein-like *Reticulomyxa* motor powers movement in both directions along the microtubule, raising the possibility that a post-translational mechanism can modify the motor's direction of movement.

These studies raise several questions about the structural aspects and mechanism of the force-generating process. The motor domains of the kinesin superfamily share considerable similarity (~ 40 per



cent identity), implying that the coupling of ATP hydrolysis to force generation requires fairly conserved features. But within the 350 amino acids there is still considerable freedom to adjust motile parameters such as velocity, torque, directionality and ATPase properties. By analogy, although most internal combustion engines work on the same principle, small changes in an engine design can dramatically affect motor performance. The structural basis for the choice of direction is particularly intriguing. It is conceivable that the location of the motor domain with respect to the rest of the protein (the carboxy terminus in the *ncd* protein and the amino terminus in kinesin) specifies the direction of movement. It is more likely, however, that subtle structural changes in the motor domain itself determine the choice of direction.

The results described here force us to re-evaluate many issues in the field of motility. Clearly, it is not possible to assign a direction and hence a potential function to a motor based on its sequence until there is a better understanding of the structural basis for directionality. In addition, the conventional explanation of force-generation by means of a large-scale conformational change in the motor seems difficult to reconcile with either the ability of a single motor to move bidirectionally (*Reticulomyxa* dynein) or two motors of similar structure (*ncd* and kinesin) to move in opposite directions. The only precedent of a bidirectional motor is the bacterial flagellar motor, a multi-protein complex that derives its energy from a proton gradient rather than ATP hydrolysis. Although thought to operate perhaps by different mechanisms, there are similarities between these prokaryotic and eukaryotic motors that we have yet to appreciate.

Could similar curiosities be in store for the myosin field? Several small myosin-like proteins (30–40 per cent amino-acid similarity) have recently been identified. Might one of these new motors move in this direction? □

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## Ways to pollen sterility

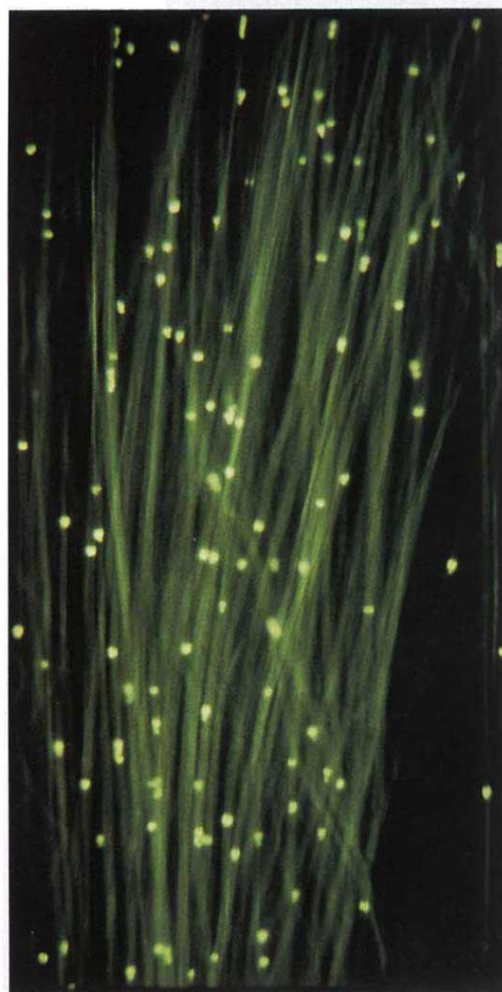
Jim Peacock

Two papers in this issue make an exciting contribution to plant biology by announcing the use of ribonuclease (RNase) activity to abort normal pollen function. On page 737, Mariani *et al.*<sup>1</sup> describe trans-

actable species, such as sunflower, tomato and brassicas. In sunflower a genetically determined system of pollen sterility makes the production of hybrid seed possible; but, in tomato and cabbage, hand emasculum of the female line is required, making seed production expensive.

Two decades ago, the hybrid corn industry did have an effective male-sterility genetic system. The sterility was controlled by DNA rearrangement in the mitochondrial genome, but it was unfortunately linked to susceptibility to a disease, southern corn blight, which in the mid-1960s obliterated a large proportion of corn production in the United States. The production of hybrid corn seed has since relied on mechanical or hand emasculum — a source of pocket money for thousands of American school children. Corn breeders have been trying to develop new male-sterility systems, either of the cytoplasmic type as existed before, or under the control of nuclear genes, but no workable system has emerged.

Mariani *et al.*<sup>1</sup> now report an entirely novel genetic method for inducing pollen failure, one which promises to be applicable to many plant species. The basis of the method is a gene construct that marries a promoter, with exquisite control, to a bacterial or fungal RNase gene. The promoter, TA29, was first described by Goldberg's group in an analysis of anther-specific genes in tobacco<sup>3</sup>, and ensures that the coding sequence to which it is attached is transcribed only in the tapetum at a particular stage of floral development. The tapetum is a



Pollen tubes of *N. glauca* growing through the style of a compatible S-genotype plant. (Courtesy of A. E. Clarke.)

genic plants carrying synthetic gene constructs that prevent pollen development and result in male sterility. And on page 757 McClure *et al.*<sup>2</sup> report on a natural system in which RNases act selectively on pollen-tube ribosomal RNA (rRNA), providing the basis of a genetically determined incompatibility system.

Plants have sex, which can be a nuisance to a seed company trying to control successful pollination in an agriculturally important crop species. Sale of hybrid corn seed is a multi-million-dollar business, for farmers prefer to plant seed from the cross of two parent lines that they know will give high-yielding F1 plants rather than seed derived from self or random pollination.

Such hybrid vigour (heterosis) is also important in several other crop and veg-

single layer of cells surrounding the pollen chamber inside the anthers and is important for pollen development. Anthers are the floral organs that normally release mature pollen for use in the pollination process.

In their new work, Mariani, Goldberg and colleagues first demonstrated that the TA29 promoter retains its specificity in transgenic tobacco plants. They linked a GUS reporter gene to the promoter and showed the integrity of both temporal and spatial gene expression. They then linked the TA29 promoter to a synthetic *Aspergillus oryzae* T1 RNase gene or to an RNase gene from *Bacillus amyloliquefaciens*. The results were spectacular — the anthers of the transgenic plants did not produce functional pollen. But apart from being male-sterile, the plants were

apparently otherwise normal, testifying to the fidelity and specificity of the promoter in the construct. Levels of tapetal-specific messenger RNA were drastically decreased, presumably preventing the production and export of proteins necessary to the developing pollen cells.

The same specificity and the same result applied when the gene constructs were introduced into oilseed rape plants (*Brassica napus*). Oilseed rape is an important agricultural crop, particularly as canola, which has seed oil with greatly reduced levels of erucic acid and seed meal lacking the anti-nutritional glucosinolate. If the new male-sterility system is to be of use agriculturally in this crop, then its reliability must first be demonstrated. Also, a component of the system that restores fertility will have to be developed so that the hybrid system can continue from generation to generation.

The authors coyly point out that there are some obvious ways to selectively restore fertility in the presence of the TA29-RNase gene, but we are left to speculate about what strategy would be used. Nevertheless, even as it stands, the work is one of the finest examples of the application of genetic engineering and recombinant DNA technology in applied plant biology.

Remarkably, RNase activity leading to the failure of pollen function has also been discovered in a naturally existing breeding system<sup>2</sup>. Self-incompatibility in plants is a process that ensures outbreeding. In general, self-incompatibility mechanisms prevent self-fertilization — pollen produced by the flowers on one plant cannot fertilize the eggs produced in the flowers on the same plant. But pollen failure sometimes results from an inability of the pollen grains to germinate on the stigma, the landing platform of the female part of a flower. In other species the pollen grains germinate, but the pollen tube is unable to grow all the way down the style to liberate the sperm cells into the embryo sac which houses the female egg cell.

Self-incompatibility systems are of two types. In sporophytic systems the recognition phenotype of the pollen grain is determined by the diploid genotype of the plant producing the pollen, whereas in gametophytic systems the phenotype of the pollen is determined by its own haploid genetic makeup.

McClure *et al.*<sup>2</sup> studied *Nicotiana glauca*, which has a gametophytic system. A diploid plant having the genotype  $S_1S_2$  at the incompatibility gene locus will produce  $S_1$  pollen and  $S_2$  pollen. The  $S_1$  pollen will not function on any female flower organell of a plant having an  $S_1$  allele in its genotype, whether it is a homozygote  $S_1S_1$  or a heterozygote  $S_1S_2$ . Similarly,  $S_2$  pollen function is negated by an  $S_2$  allele in the female genotype.

In *N. glauca*, the growth of the pollen

tubes of incompatible pollen is arrested in a particular zone of the style, whereas compatible pollen tubes grow through this zone and bring about fertilization. The  $S$  alleles code for glycoproteins<sup>3</sup> found in the stylar tissue that were recently shown to be ribonucleases (S-RNases)<sup>4</sup>. This finding led to speculation as to whether the S-RNases are the 'killer' molecules in the natural self-incompatibility system.

McClure *et al.* now demonstrate that RNA is degraded in incompatible pollen tubes, whereas there is no RNA degradation in compatible pollen tubes. RNA degradation appears to be restricted to ribosomal RNA, with poly(A)<sup>+</sup> RNA and transfer RNA not being affected. This  $S$ -allele specificity of the breakdown of pollen RNA implies that the S-RNases of the style can be selectively active inside the pollen-tube cell. Tests *in vitro* did not provide evidence for any  $S$ -allele rRNA specificity of the S-RNases, which attacked rRNA of heterologous species as well as that of *N. glauca*.

#### SOLAR PHYSICS

## Looking for relationships

Michael F. A'Hearn

IN recent years there has been considerable interest in the relationship between comets and asteroids, a question studied extensively by Bill Hartmann and various collaborators. In his latest foray into this area<sup>1</sup>, Hartmann, together with Tholen, addresses the question of whether cometary nuclei develop inert mantles that choke off the vaporization that gives comets their familiar appearance, leaving an inert body which we would then identify as an asteroid. This has been suggested as a second origin for the Earth-approaching asteroids, the first being the region of the asteroid belt near the 3:1 orbital resonance with Jupiter. The possibility is widely accepted, although it is still uncertain what fraction of Earth-approaching asteroids have been produced in this way and there is considerable disagreement over which Earth-approaching asteroids might be extinct nuclei.

The relationship between comets and asteroids also involves the question of whether the original condensation of solid material in the early solar nebula led to a continuous variation in the physical properties of the early planetesimals, such that the cometary nuclei were just an extension of the distribution of asteroid types. Just ten years ago, Gradie and Veverka<sup>2</sup> proposed that the non-volatile material in cometary nuclei might be similar in composition to the material seen on the surfaces of the dark, reddish asteroids (then called RD-type for red-dark but now called simply D-type) that dominate the outer asteroid belt. They suggested that

So the puzzle of  $S$ -allele specificity remains, but the scope of the problem is narrowing. Is there selective entry of S-RNases into incompatible pollen tubes or are the S-RNases selectively muzzled in the cytoplasm of compatible pollen tubes? Either way we still have no direct molecular clues as to the role of the  $S$  alleles in the male gametophyte, and yet the breeding system clearly dictates that they must be responsible for recognition and interaction of male and female tissue. Could the  $S$ -gene locus encode a polypeptide that functions differently in male and female cells? □

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this material is organic, but there are still no diagnostic data to show this to be the case for dark asteroids, although the missions to P/Halley showed unequivocally the presence of organic material in that comet. Since that paper<sup>2</sup> appeared, Hartmann and his collaborators have demonstrated the many similarities between cometary nuclei and various groups of asteroids in an attempt to establish a genetic relationships between them.

Hartmann and Tholen<sup>1</sup> now argue that cometary nuclei have a distribution of shapes most like that of the Trojan and Hilda asteroids, the most distant dynamically stable groups of asteroids. The brightness of all these bodies varies, over short periods, much more than in most other groups of asteroids. The variation in brightness is generally attributed to the varying cross-section that would be presented by a prolate object rotating about its short axis, so that the larger amplitudes imply that the Trojan asteroids and cometary nuclei are more prolate than asteroids in the main asteroid belt. Hartmann and Tholen use this similarity not to infer a direct genetic relationship between the cometary nuclei and the distant asteroids, but rather to suggest that parallel evolution has occurred, in which devolatilization leads to an increase in the elongation of the object. This implies that the Trojan and Hilda asteroids once contained substantially more volatile material than they do now and may have had a cometary appearance. Whether these volatiles were similar to



the ices now in comets is not specified. The elongation mechanism, some versions of which have been discussed by other authors, is certainly physically plausible.

There are, of course, dangers in building theories out of such similarities: some other object not considered may have a closer resemblance; the differences may outweigh the similarities; and any single similarity may be purely coincidental. In this case, although the connection with Trojan and Hilda asteroids is intriguing, there seems to be a closer similarity between cometary nuclei and small asteroids than between cometary nuclei and the Trojan and Hilda groups of asteroids. Binzel *et al.*<sup>3</sup> have shown that the mean amplitude of brightness variation for small main-belt asteroids (average diameter 11 km) is 0.33 magnitudes (about 35 per cent), and for small Earth-approaching asteroids (average diameter is 3 km), after correcting for an observational bias towards larger amplitudes, the mean amplitude is 0.39 magnitudes (43 per cent). These two values are significantly larger than that for the much smaller sample of 'small' asteroids used by Hartmann and Tholen, as well as being significantly larger than the average for their sample of Trojan asteroids. The values are, in fact, the closest matches to the mean amplitude for the cometary nuclei of any groups of asteroids. This would suggest that the most appropriate connection between cometary nuclei and asteroids is through their size rather than through the site at which they formed, as small asteroids probably originated throughout the belt. The devolatilization proposed by Hartmann and Tholen would, of course, be more effective on smaller bodies which have a preponderance of surface material.

A real problem in such studies is obtaining data for a sufficiently large sample of cometary nuclei, as these objects are notoriously difficult to observe and there are strong selection effects in the observed sample. As Hartmann and Tholen note, there were only seven nuclei in their original sample (although they arbitrarily added an asteroid to their graph using the partly circular reasoning that its dynamics resemble those of a comet). The unequivocal verification that the minor planet Chiron has a cometary nature (see below) provides a datum that quite changes one's idea of the comet distribution, reinforcing the correlation with size.

Meanwhile, the approach of Hartmann and his collaborators has recently been adopted by Jewitt and Luu<sup>7</sup> who have compared optical spectrophotometry of cometary nuclei and Trojan asteroids, much as Hartmann, Tholen and Cruikshank<sup>5</sup> previously compared the colours of cometary nuclei with those of asteroids identified as possible extinct comets. Using new spectrophotometric data,

Jewitt and Luu come to essentially the same conclusion, namely that the asteroids are dark and vary between slightly reddish and red and that the spectrophotometric properties of cometary nuclei fall in the same range. However, this range encompasses several different taxonomic classes of asteroids and the spectral range used to obtain the data was chosen primarily for the availability of equipment rather than for its diagnostic capability. Jewitt and Luu were limited to two cometary nuclei for which they had data, and Hartmann *et al.* were limited to data on three cometary nuclei, one having a much higher albedo than is typical of the dark asteroids.

The question remains, therefore, as to whether or not there really is a genetic relationship between the asteroids and the comets. There is certainly a genetic relationship in our perception of these bodies. This is exemplified by the evolving description of Chiron, which was first called 'slow moving object Kowal' in honour of its discoverer Charles Kowal, then characterized as the prototype of a new group of asteroids between Saturn and Uranus, and most recently was recognized for what it is, truly a comet exhibiting a coma of dust and (intermittently) of gas<sup>6-8</sup>. This cometary nucleus also exhibits a spectral reflectivity in the range of that of dark asteroids. An evolution of our understanding coupled with an incorrect initial classification, however, should not be mistaken for evidence of any genetic or evolutionary relationship between Chiron and asteroids. A recent analysis of the orbital evolution of Chiron by Hahn and Bailey<sup>9</sup> shows that there is a reasonably high probability that Chiron approached much closer to the Sun in the past so that its surface might be typical of highly evolved cometary nuclei such as those proposed to have evolved into Earth-approaching asteroids. Nevertheless, this can only be considered suggestive at best. It is clear that more effort is required to devise diagnostic measurements that can be made on both cometary nuclei and asteroids to show some compositional similarity. Evidence of spectral absorption features common to both would go a long way towards showing some common elements in their past evolution. □

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## Point of recall

A SMALL barbed or asymmetric foreign object can be ratcheted through our tissues by its own ceaseless movements. Last week Daedalus devised a surgical micro-needle to be manoeuvred within the body on this principle, steered by high-frequency vibro-massage pads applied to the skin. He now plans to use it on the brain itself.

Surgeons have learnt a great deal about the brain by operating on it under local anaesthetic. When searching for the causative site of an epilepsy, say, or muscular dystonia, they often find specific regions which, when touched or electrically stimulated, set off specific moods in the patient, or excite particular memories. Daedalus's micro-needle will take this process out of the operating theatre and into everyday life. Once the needle has been inserted into the brain cavity, the patient himself will be able to move it around his brain by means of a special vibro-hat. Too blunt and slow to puncture or damage the cells, the needle will nose its way harmlessly among the neurons and nerve fibres. By switching on a low-frequency magnetic field, the patient can induce currents in the needle to stimulate whatever site it has reached.

Thus the whole internal architecture of consciousness will be revealed. If a stimulated site stores a specific mood, the patient will be swept away by it; if an action, he will carry it out; if a memory — even long-forgotten — it will come flooding back in full detail. If the brain really does record everything, the whole of our past life will be accessible at last. The brain may even lay down its memories in spatial diary order, so that the needle can be stepped sequentially through them. Whole sections of life could then be relived for instant autobiography or nostalgic *recherche du temps perdu*.

Psychiatry, of course, will be transformed. Childish memories, repressed emotions, forgotten dreams, all could be dialled up almost to order without wasting months and years on the couch. Even cure (long a dirty word among psychoanalysts, who tend to repress their inadequacies) may at last be possible. If the patient really is dogged by traumatic childhood memories, they could be tracked down by the internal needle, identified, and then erased by boosting the frequency and strength of the investigative magnetic field until induction heats the needle to cauterizing temperatures. Oedipal conflicts and penis envy could likewise be located and burnt away. Obsessive guilt, feelings of inferiority, hopeless longing, irrational fears, compulsive actions, all could neatly be cut out of patient's psyche without affecting anything else. He could even do it himself, tailoring his internal world to his own needs without surrendering his autonomy to the medical establishment.

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# Recipe for safer sauces

SIR—Contamination of intact chicken eggs by *Salmonella enteritidis* has led to a notable rise in food poisoning in the West<sup>1-4</sup>. Health authorities have named home-made mayonnaise, hollandaise and béarnaise sauces as dishes that pose some hazard<sup>5</sup>. Mayonnaise, prepared by emulsifying vegetable oil in raw egg yolks, has been implicated in several outbreaks of salmonellosis, including one at the House of Lords<sup>6</sup>. The acid ingredients in this sauce can eliminate salmonellae from raw yolks, but only over a period of hours to days<sup>7</sup>. Hollandaise and béarnaise sauces, butterfat-in-water emulsions thickened with lightly cooked yolks<sup>8</sup>, are usually heated briefly to 70 or 75 °C, but some salmonellae from an initially large population may survive this treatment<sup>9,10</sup>. Egg yolk seems to increase the resistance of salmonellae to thermal killing<sup>11</sup>.

The standard kitchen method for minimizing microbial contamination, thorough boiling, has not been considered applicable to emulsified sauces. Egg yolks harden and emulsions break well below 100 °C, so cooks never intentionally boil either the yolks alone or a finished sauce. But a sufficiently low pH can delay or prevent the heat coagulation of egg proteins<sup>8,12</sup>. Under suitable biochemical and thermal conditions, egg yolks can be boiled without curdling them or fatally compromising their ability to produce smooth, stable emulsions.

The method is as follows. Raw egg yolks are mixed thoroughly with an equal volume of water and from one-third to an equal volume of lemon juice or vinegar. This mixture is then placed in a small glass bowl, covered and irradiated in a microwave oven at maximum power until it bubbles; for 1- and 2-yolk mixtures, 1 minute or less, depending on oven power. The unevenly heated mixture is then beaten with a fresh implement and cooked a second time until it has bubbled for 5 to 10 seconds. Beaten with another fresh implement as it cools down, the yolk mixture retains the consistency of a stirred custard. I find that this mixture can then be used to make an acceptable if slightly more heat-sensitive hollandaise or béarnaise sauce or, as long as unrefined olive oil is only a small fraction of the total oil volume, a stable mayonnaise.

To test the effectiveness of this method of eliminating large bacterial populations, a culture of *S. enteritidis* strain 1601E, phage type 4 (B. A. D. Stocker, Stanford University), grown in broth with shaking for 18 hours at 37 °C, was inoculated into egg yolks at about  $5 \times 10^8$  colony-forming units per millilitre of yolk. The yolks, initial pH 6.2, were then mixed with water and one-third their volume of either lemon juice or vinegar; the final pH of the

mixtures were 4.0 and 4.8, respectively. After an initial sample was removed, the yolk mixtures were treated as above in a Quasar microwave oven rated at 600 W, and sampled again. Bacteria were estimated by dispersing yolk samples in saline and plating dilutions on standard media. Recovery of colony-forming units from the yolks sampled before heating was consistent with the known inoculum. No live bacteria were detected in samples taken after the second heating period. The surviving population, if any, thus was probably smaller than 5 cells per millilitre of yolk.

These results suggest that cooks can greatly reduce the health risk posed by yolk-based sauces. If public-health laboratories would verify that the combination of acidification and rapid boiling reliably eliminates salmonellae from yolks, then this method could provide a more palatable alternative to abstention from freshly made mayonnaise, hollandaise and béarnaise sauces<sup>5</sup>.

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## Radiation and Down's syndrome

SIR—Recent epidemiological evidence<sup>1</sup> reported in *Nature*<sup>2</sup> has suggested that paternal occupational exposure to repeated low doses of ionizing radiations may be responsible for a tenfold excess of leukaemia in children. It has been pointed out<sup>3</sup> that, according to such an explanation, the postulated inheritable lesions determining leukaemia would be induced at a high rate, while no other genetic effect has been noticed in the progeny. Therefore, detection of those lesions in the exposed parents and in the progeny should provide the crucial epidemiological evidence and eventually some clue concerning their peculiarly high frequency and/or heritability. Otherwise, parental exposure to ionizing radiation might also be considered as an indicator of children's

exposure to the same or to another leukaemogenic factor.

In a similar context, we have evaluated<sup>4</sup> the effect of cumulative parental exposure to abdominal diagnostic X-rays on non-disjunction of chromosome 21, as detected by a trisomic conceptus (Down's syndrome). Nondisjunctional parents have been defined as cases and their partners as controls, avoiding recall bias by applying an obvious double-blind interview concerning exposure. A significantly increased risk for nondisjunction has been detected in exposed fathers which, not surprisingly, is correlated with age. Results observed in exposed mothers are consistent with a bifactorial aetiology, ageing and exposure to ionizing radiation, acting according to an additive model.

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## New tricks for old dogmas?

SIR—Gehring *et al.* suggested<sup>1</sup> a possible role of cytoplasmic calcium ( $[Ca^{2+}]_{cyt}$ ) in higher plant tropisms. In our opinion, some physiological aspects of this work were less than ideal. Furthermore, the authors seemed to be guided in the interpretation of their results by some old dogmas, rather than considering contemporary work.

None of the cellular responses reported by the authors can be unambiguously linked to a tropic response. In the case of the phototropism experiments, white light was used and no evidence was presented that the changes in  $[Ca^{2+}]_{cyt}$  were not the result of red or blue light acting non-phototropically (both known to occur in coleoptiles). In the case of the gravitropism experiments, the unilateral blue illumination to the lower side (due to the laser used as part of the measurement technique) could have produced a physiological response. One reason for questioning the relationship of the observed changes in  $[Ca^{2+}]_{cyt}$  to any tropic response is the fact that the main changes in  $[Ca^{2+}]_{cyt}$  observed after unilateral white-light treatment were at the shaded side, with no changes reported at the illuminated side. This is



anomalous because the most consistent change in growth rate in phototropism usually occurs at the illuminated side where growth virtually ceases<sup>2</sup>. If there is no detectable change in  $[Ca^{2+}]_{cyt}$  at the illuminated side then there is obviously no correlation between  $[Ca^{2+}]_{cyt}$  and cell extension.

Surprisingly, Gehring *et al.* state that the observed changes in  $[Ca^{2+}]_{cyt}$  occur only if the apex of the coleoptile is illuminated; they claim that this is consistent with the known site of perception of the phototropic signal. Unfortunately, this old misconception about the site of perception keeps cropping up despite the fact that it has been shown<sup>3</sup> that a phototropic signal can be detected by all growing regions of coleoptiles. If the changes in  $[Ca^{2+}]_{cyt}$  are really involved in phototropism, they should be looked for in decapitated coleoptiles and in coleoptiles subjected to unilateral illumination at any place in the elongation zone.

Gehring *et al.* used the Cholodny–Went model of tropisms and ignored the many experiments conducted over the past two decades which question the validity of this model — for instance, Went's original experiments have recently been repeated using modern analytical methods<sup>4</sup>. Contrary to Went's original claim, the flow of auxin from the illuminated and shaded sides of phototropically stimulated coleoptile tips are identical. Gehring *et al.* also ignore the careful analyses of Weiler and co-workers<sup>5</sup> which have failed to detect any significant tropistically induced changes in auxin concentration during gravi- or phototropism. Finally, they still seem to believe that auxin movement across tropistically stimulated organs is relevant despite the fact that the two isolated halves of longitudinally bisected organs show a gravitropic response<sup>6</sup>. The fact that tropic responses can occur in the absence of auxin redistribution and that, even when auxin redistribution does occur, it seems slower than the reported changes in  $[Ca^{2+}]_{cyt}$  should alert Gehring *et al.* to the possibility that auxin does not mediate the changes they report.

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GEHRING *ET AL.* REPLY—The Cholodny–Went model has been extensively supported for phototropism in maize, the material used in our study (see, for example, refs 7,8). Although a definitive study measuring auxin levels remains to be done, the extant evidence for maize does favour a lateral redistribution of auxin during phototropism<sup>7,9</sup>. Therefore, our use of the model is appropriate.

For phototropism in general, the “constantly observed growth inhibition” on the irradiated sides of various species of

bending stems usually results from non-tropic light–growth responses being simultaneously induced along with the phototropic response<sup>10</sup>. By contrast to maize, where the phototropic response resembles what is known for that of many other plants<sup>7</sup>, the phototropic response of *Avena* seems to be unique in several respects: it is the only plant in which a region of negative curvatures occur in the phototropic fluence response curve<sup>11</sup>; the pattern of growth response is apparently more varied than in other plants<sup>2</sup>; and measurements of auxin concentrations in oat have given confusing and contradictory results<sup>9</sup>. Therefore, oat would seem a poor model system for phototropism. Further, problems in applying the model to oat are irrelevant as we studied maize.

On the technical points raised by Firn and Digby, we accept that the use of blue light as an inductive stimulus would have been preferable to white light. Nevertheless, we are confident that the observed  $[Ca^{2+}]_{cyt}$  and  $pH_{cyt}$  effects are specific for the phototropic response. In upright coleoptiles illuminated with the laser beam only, no changes in  $[Ca^{2+}]_{cyt}$  and  $pH_{cyt}$  occurred: such changes were observed only when phototropic bending was induced, hence our conclusions that these effects are specific for phototropism.

Firn and Digby have promulgated a critique of the Cholodny–Went model in which they demonstrated several areas where evidence in its favour was either lacking or unconvincing<sup>13</sup>. Their analysis has spurred considerable investigation, which has in many instances been supportive of the model, especially for maize<sup>7</sup>. We suggest that this is a rare instance of being able to teach an old dogma new tricks.

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## Air pressure and methane fluxes

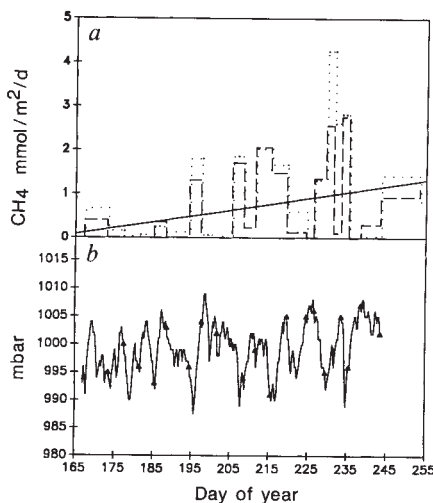
SIR—The increase in the atmospheric concentration of methane gas<sup>1,2</sup> is an important factor in the analysis of global warming trends. At Mirror Lake, New Hampshire, we observed that sporadic methane bubble releases (ebullition) from the sediments were correlated with changes in local air pressure. This is the first time such a correlation has been reported. Low-air-pressure events usually associated with storm systems appeared to induce ebullition (18% increase per millibar drop in air pressure), whereas high pressure inhibited ebullition.

Mirror Lake is shallow (11 m) and unproductive, yet has thick organic sediments and becomes anoxic below 9 m in depth by mid-summer<sup>3</sup>. Here we discuss only deep sites in the hypolimnetic zone (see ref. 4 for additional details and methodology). Briefly, rising gas bubbles were collected continuously during the summers of 1987 and 1989 in large inverted funnel traps<sup>4,5</sup>. Gases were removed every 2–6 days and transferred immediately to the laboratory for analysis by dual-column TCD gas chromatography. The loss rate of methane from the traps was approximately 3% per day.

During 1987, methane ebullition rates were erratic, with an average rate of 0.76 and a high of 4.26 mmol m<sup>-2</sup> per day at the deepest sites (10 m). The proportion of methane in the bubbles was relatively constant 70 ± 7%. Ebullition rates and hypolimnetic temperatures increased during the summer, until 6 October 1987 (day 279), when autumn circulation occurred in the lake, and the hypolimnion was re-oxygenated. All sites, including shallow-water sites, showed a remarkable synchrony in bubble release during 1987. We observed many bubbles rising to the surface of the lake on some days and none on others. The ebullition rates at two sites with the longest continuous records correlated highly with each other ( $r^2 = 0.71$ ,  $a < 0.01$ ,  $n = 42$ ), and showed large deviations from the regression of ebullition versus day of the year ( $a$  in the figure).

We examined several factors which might influence ebullition, including wind speed, water temperature, solar radiation, lake level, local water table and air pressure ( $b$  in the figure). Only the lowest air pressure in each sampling period explained a large and significant amount of the residual variation in ebullition rate at the two deepest sites ( $r^2 = 0.42$ ,  $a < 0.01$ ,  $n = 42$ ) with ebullition increasing 18% (0.14 mmol m<sup>-2</sup> per day) for each millibar drop in pressure.

In 1989, ebullition was again related to the lowest air pressures ( $r^2 = 0.22$ ,  $a < 0.05$ ,  $n = 26$ ). During a 64-day period,



**a**, Methane ebullition rates at two sites (10-m depth) during 1987 in Mirror Lake, New Hampshire. Dashed vertical bars, rates and the sampling intervals versus day of the year. The line is the best-fit regression of the rate versus time. **b**, Associated air pressure (J. Zabranski, unpublished data). Triangles, beginning and end of the sampling periods.

more than half the methane flux occurred during two sampling intervals in only 10 days. These two periods of rapid methane ebullition corresponded to the two lowest air-pressure events of the summer: the first (985 mbar) occurred with a heavy rainfall on 5 August, the second (982 mbar) was associated with the passage of (former) hurricane Hugo on 23 September.

The change in air pressure we measured was only 1–3%, yet we believe this change directly affected the volume of the gas bubbles in the sediments. Observations of pores on the lake bottom suggest<sup>6</sup> that bubbles within the sediments occupy vertical 'bubble tubes'. When pressure falls, expansion of the bubbles is limited to the vertical, resulting in bubble release. Others have noted an apparent hysteresis effect on ebullition<sup>7</sup>, which implies that flux rates may not be a simple linear function of pressure changes.

Highly sporadic, yet synchronous ebullition has also been reported in other sediments<sup>6–11</sup>. There are also reports of gas

release from soils being triggered by low-pressure events<sup>12</sup>. As most studies do not sample ebullition continuously, they may not sample the main ebullition events, particularly when sampling tends to occur during fair weather (at high air pressure). Attempts to reduce the sampling variability by extending the collection interval to longer periods (say weeks) may result in serious underestimates due to diffusion and/or oxidation losses in the traps.

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## Flight of the bumblebee

SIR—The letter by C. P. Ellington *et al.* (*Nature* 347, 472; 1990) on the flight of bumblebees has stimulated me to resurrect my own answer to the problem of bumblebee flight, put forward in a paper that was rejected by the *Journal of Experimental Biology*, I think in 1950.

The theoretical difficulty in understanding how a bee can fly, and in particular how it can hover, is as follows. A hovering insect supports its weight by projecting downwards a jet of air. Then  $W = mv$ , where  $W$  is the weight of the insect,  $v$  is the velocity of the jet and  $m$  is the mass of air traversing the jet in unit time. Clearly,  $m = \rho av$ , where  $\rho$  is the density of air and  $a$  is the cross-sectional area of the jet. Knowing the weight and wing span, one can estimate  $a$  from the standard theory of aircraft propellers, and hence can estimate  $v$ . The kinetic energy added to the air in unit time is  $mv^2/2$ .

The snag is then as follows. Comparing this estimate of the necessary work being done with estimates of the rate at which sugar is used by a tethered flying insect suggested an overall efficiency that approached 100 per cent, which seemed implausible.

My fellow undergraduate at University College London, M. J. Davis, and I therefore decided to measure the direction and velocity of the airflow round a tethered flying insect. We used hoverflies because we found bees reluctant to fly when tethered. We surrounded the insect with particles of metaldehyde and photographed it with a flashbulb, timing the flash with a strip of bromide paper on the rim of a gramophone. The resulting photographs were fairly awful by modern standards, but were good enough to show that the velocity of the air in the jet was about one-third of the theoretical value, and the area of the jet correspondingly

greater. Because of viscosity, the air that actually passes through the disk swept out by the wings drags along with it a substantially larger volume of air. The answer to the conundrum of bumblebee flight is that, at low Reynold's number, the bee handles a larger volume of air than its small wing span might suggest.

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## Meaning of life

SIR—In his paper<sup>1</sup> entitled "How Long Do Neutrons Live?", Freedman does us all a service by collating and assessing current estimates of neutron lifetime. His conclusion, that the weighted mean of the various estimates of lifetime is  $887.6 \pm 2.7$  seconds, is given to three significant figures, but unfortunately some readers may have a significant doubt as to what quantity is actually being enumerated.

Dictionaries define lifetime as "the time during which an individual lives". The decay of neutrons in free space being a random process, it follows that Freedman's result cannot apply to the lifetime of any individual neutron. It must, therefore, be either the mean-life or the half-life. But which?

A survey of colleagues in this department showed that a number of them were unsure, their conclusion probably being based on past experience. Those of us reared on earlier editions of the *Handbook of Chemistry and Physics*<sup>2</sup> know of a column labelled 'lifetime' in the table of isotopes. The explanation to the table defined 'lifetime' as being 'half-life'. Some time between 1975 and 1987, the editors changed the column label to the totally unambiguous 'half-life'. On reading Freedman's paper my first reaction, and that of some others also, was to believe that the latest estimates of neutron half-life were longer than those previously proposed. In fact, Freedman reports an improved value for the mean lifetime. The usage of lifetime to define this quantity may be clearly understood in the particle-physics community, but is not necessarily clear to the wider audience to whom a result of this type could well be of considerable interest.

The difference between mean-life and half-life is a factor of 0.693. It is important that authors make it clear which of the two they are reporting.

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# Natural gas and the greenhouse

SIR—Wallis in a recent Scientific Correspondence<sup>1</sup> claimed that use of natural gas instead of coal in power stations will lead to 0.8 to 3.0 times the emissions of greenhouse gases. His argument is flawed by an arithmetical error in the treatment of leakage of natural gas, and by his failure to realize that such incremental uses will not lead to any significant increase in natural gas leakage.

Wallis uses the concept of relative carbon coefficients of 0.43 for natural gas, 0.62 for oil and 0.75 for coal<sup>2</sup> but does not give the units: they are gigatonnes (Gt) of carbon per terawatt year (TW yr) energy (1 TW yr =  $31.536 \times 10^{18}$  J). He takes natural gas losses of 3–10% of final use, assumes 75% methane content, multiplies by 25 to take account of the enhanced infrared absorption of atmospheric methane compared with CO<sub>2</sub> and by  $\eta$  the ratio of their lifetimes. He then adds the

oil production<sup>4</sup>. Attributing 40% to gas production gives a loss rate of less than 0.4% on a gas-supplied basis. Fourth, methane from coal mining is given by Eyre<sup>5</sup>, quoting British Coal, as  $340 \text{ g GJ}^{-1}$ , which is equivalent to  $12.5 \text{ m}^3 \text{ t}^{-1}$ .

Finally, it is widely accepted that the lifetime of the methane molecule in the atmosphere is 10 years and its effectiveness compared with CO<sub>2</sub> is about 25. The appropriate lifetime for CO<sub>2</sub> is much more contentious but the effect of anthropogenic emissions appears to be long-lived. Derwent<sup>6</sup> has analysed the relative effectiveness of methane and CO<sub>2</sub> over various time horizons, taking into account CO<sub>2</sub>, ozone and stratospheric water vapour resulting from the atmospheric destruction of methane. Converting Derwent's results from mass into moles gives the relative effect,  $\alpha$ , at time  $t$  from emission of 1 mole of each at  $t = 0$ . This

|                                               |      |      |      |      |
|-----------------------------------------------|------|------|------|------|
| 1 Time horizon (years)                        | 20   | 50   | 100  | 500  |
| 2 $\alpha_t$                                  | 30.5 | 16.8 | 10.5 | 4.4  |
| 3 $G_{\text{gas}}: G_{\text{coal}}$ 3.5% loss | 0.86 | 0.75 | 0.69 | 0.63 |
| 4 $G_{\text{gas}}: G_{\text{coal}}$ 1.0% loss | 0.55 | 0.56 | 0.57 | 0.57 |
| 5 Relative emissions per kWh (e) for 1% loss  | 0.47 | 0.47 | 0.48 | 0.48 |
| 6 Break-even loss %                           | 4.6  | 6.8  | 9.6  | 20.2 |

quantity 0.75 (0.03 to 0.1)  $25\eta$  to 0.43 as an absolute quantity instead of as a proportion, thus increasing the apparent effect of leakage by  $1/0.43$ . Neglecting the emission of methane from coal mining he derives the relative greenhouse effects  $G_{\text{gas}}: G_{\text{coal}}$  as 0.95 to 4.1. Correcting the arithmetic gives 0.73 to 2.1.

Wallis uses  $20 \text{ m}^3 \text{ t}^{-1}$  to make allowance for mine methane and gives a factor of  $0.21\eta$  to add to the carbon coefficient. It is not clear how he derives this factor:  $20 \text{ m}^3$  contains about 832 moles of methane. The calorific value of power-station coal is about  $24.5 \text{ GJ t}^{-1}$ , leading to  $0.321\eta$  Gt carbon equivalent per TW yr.

On this basis the final result should be

$$G_{\text{gas}}: G_{\text{coal}} = \frac{0.43 (1 + 0.75 (0.03 \text{ to } 0.1) 25\eta)}{0.75 + 0.32 \eta}$$

giving the range 0.61 to 1.31.

The above equation accepts all Wallis's assumptions, but many of these are at least questionable. I suggest the following alternatives. First, natural gas in the United Kingdom contains about 93% methane. Second, unaccounted-for gas includes financial losses due to undercharging (declared calorific value is less than actual; pressure and temperature factors generally favour the customer) and theft as well as leakage losses. For the sake of argument (only), unaccounted-for gas will be taken as an upper limit for leakage and put at 3%.

Third, off-shore venting of methane has been given as 270 kt for the United Kingdom<sup>3</sup>, with the main part relating to

factor would replace  $25\eta$  in Wallis's treatment. Its use leads to the results in line 3 of the table, which demonstrate the generally low emissions for gas compared with coal.

Wallis's claim relates specifically to power generation. Gas supplies to power stations will be from welded high-pressure steel pipes, and the gas will not pass through the low pressure system where most losses occur. A very generous allowance for losses would be 1%, giving the relative emissions per unit of input shown in line 4 of the table. The relative emissions per unit of electrical output are shown in line 5 — taking 45% conversion efficiency for gas in combined cycle compared with 38% for coal in new conventional units with flue-gas desulphurization. The conclusions, using Wallis's argument but more reasonable assumptions — several of which are more favourable to coal than his — show the opposite of his claim. Natural gas gives only about half the greenhouse emissions of coal in power generation. As a bonus, the sulphur

emissions from gas firing would be only 1% of those from the coal plant.

Finally, gas losses from the low-pressure system are governed by pressure rather than throughput. New loads will not necessarily incur extra losses; all new pipes are laid in polyethylene or steel and are essentially leak-proof. The existing cast-iron mains are being progressively replaced.

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WALLIS REPLIES—Several relevant data have been published since I conjectured<sup>1</sup> that methane leakage from gas fuel production and distribution may tip the relative greenhouse balance in favour of coal combustion. James legitimately criticizes my first estimate of the 'greenhouse' ratio for electricity generation by gas compared with coal combustion of 0.8–3.0 and claims values of 0.55–0.86. But he does not comment on my revised detailed estimate of 0.53–2.0, including new data, available as a University of Wales, Cardiff preprint (March 1990).

One difference is that leakage from North Sea gas wells is probably much higher than the unauthenticated 0.34% James cites from UK Department of Energy sources. The 3% upper limit for land-based leakage is disputable, as British Gas reported 4.5% "unaccounted for" gas in 1986, more than half their pipework is pre-1969, designed for lower pressure town (CO) gas, and leakage from their pumping, pressure-reducing and conditioning plant is additional. The third main difference arises through disputing the box-diffusion model of the CO<sub>2</sub> cycle used by Derwent<sup>6</sup> that gives 'components' as long-lived as 815 years or even thousands of years<sup>7</sup>. Nevertheless, it is reassuring that his result lies within the revised range of 0.53–2.0 and, as expected, comes at the low end — but the verdict is still not proven.

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JAMES REPLIES—Wallis's new data use a multiplier of up to 250 for methane which assumes a lifetime for CO<sub>2</sub> of 7 years; an enhanced infrared absorption of methane of 70 times CO<sub>2</sub> to allow for degradation products; and a reduction in the effectiveness of CO<sub>2</sub> by 60% to allow for a biological feedback.

Derwent's treatment of the effect of methane has been accepted by the Inter-governmental Panel on Climate Change but with factors 25% lower than those used here. Further, British Gas does not report unaccounted for gas. □

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# No place like hospital?

Alison Macfarlane

**Safer Childbirth? A Critical History of Maternity Care.** By Marjorie Tew. Chapman and Hall: 1990. Pp.328. Pbk £14.95, \$19.95.

"SEEING how destitute of comforts, means and medical appliances many women are, the thought occurred to some benevolent person that they might be received and delivered in hospitals. . . . Contrary to expectation the advantages these institutions offered were overbalanced by one dread drawback; the mortality of mothers was not diminished; nay it became in some instances excessive; in other instances appalling."

When William Farr wrote these words in the Registrar General's annual report for 1870, fewer than 1 per cent of women in England and Wales gave birth in 'lying-in' hospitals or workhouse infirmaries. A century later, when the Peel committee reported its views on the future of hospital and domiciliary maternity services, only 13.5 per cent of women gave birth outside hospital. The committee concluded that "We consider that the resources of modern medicine should be available to all mothers and babies, and we think that sufficient facilities should be provided to allow for 100% hospital delivery. The greater safety of hospital confinement for mother and child justifies this objective."

A decade later, only 1.3 per cent of women delivered outside hospital, and the figure remains at about 1 per cent. Edwina Currie reiterated the English Department of Health's policy in 1987: "we aim to minimise the risks to babies by encouraging delivery in hospital preferably with access to the full range of facilities which are likely to be found on district general hospital sites." Her statement went on to claim that the fall in perinatal mortality between 1979 and 1985 had resulted from this policy.

An early critic of this logic was the epidemiologist Archie Cochrane. In his now classic book *Effectiveness and Efficiency*, published in 1972, he pointed out that the only quantitative evidence given for the claim in the Peel committee's report was a table showing decreasing maternal and perinatal mortality rates and an increasing proportion of deliveries in hospitals over the years 1955-68. The implication was that the latter had caused the former yet, as Cochrane pointed out, "there have

been other downward trends in mortality which were unrelated to medical interference, for example, tuberculosis before 1948". Cochrane recast the committee's table, replacing hospitalization rates with the decreasing mean length of stay in hospital, and observed: "One could as wrongly or rightly conclude from this that the shorter the stay the lower the mortality,

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Home or away? The debate continues over whether place of delivery affects perinatal mortality.

as that the higher the hospitalization the lower the mortality."

Marjorie Tew has been the most longstanding and persistent challenger to UK government policy on place of delivery. She "stumbled into the subject" in 1975 while working in the Department of Community Medicine at Nottingham University. To her surprise, she found that routine statistics did not support the prevailing orthodoxy. After she tried to publish her analysis, her contract was not renewed. She moved to a part-time job in another department, but continued her analyses in her spare time in what she describes as a "dogged, lonely campaign".

*Safer Childbirth?* brings together material from Tew's articles, providing her perspective on the changes from home to hospital birth. She describes changes in the status of the professions working as birth attendants, charting the declining status of midwives and the rise in the

power of obstetricians, the introduction and development of antenatal care and the increasing use of obstetric intervention in labour and delivery. She also discusses public concern about maternity care as expressed in the activities of government and parliamentary committees and of voluntary organizations.

Tew then presents statistical analyses of mortality and morbidity associated with delivery in hospitals with obstetric units, in general-practitioner maternity units and at home. Opening with an explanation of mortality rates and the methods used to standardize them, she discusses methods of evaluation and the construction of risk scores. She uses these to review data about the mortality of mothers

and babies derived from routine sources, confidential enquiries and the special British birth surveys of 1958 and 1970, and discusses some data from other developed countries. She looks at the two central assumptions — that the safest place for birth is in a hospital with a consultant obstetric unit, and that the switch to this practice has been responsible for the fall in perinatal mortality.

Looking in much greater detail than Cochrane, Tew shows that there is no statistical correlation between regional perinatal mortality rates and the percentages of births in hospital, and that there is a similar absence of correlation between year to year changes in these figures. In addition, she shows a lack of correlation between changes in mortality and those in induction, forceps delivery and rates of caesarean section.

As a result, she is on firm ground in arguing that the rise in hospital birth and obstetric intervention is not responsible for the reduction in perinatal mortality. She is on less firm ground, however, in arguing that the reduction in mortality has arisen solely because of the improved health of women as a consequence of better nutrition and housing, and that changes in patterns of care have made no contribution whatsoever. This may be true, but there are no adequate data to allow the relative contributions to be assessed.

Tew bases her argument on her second conclusion, that consultant obstetric units are much less safe places for giving birth than home or general-practitioner units. There is no doubt that institutional births were unsafe in the past, because of the risk of infection from puerperal sepsis. Further, in the 1920s and 1930s, women who paid possibly inexperienced general practitioners to deliver them in small cottage hospitals may well have put

Many Evans



themselves at higher risk than women who gave birth at home with an experienced midwife. By the mid-1930s, however, the decline in severity of puerperal sepsis, which seems to have coincided with the discovery of a cure in the form of sulphoamide drugs, changed the picture dramatically. In the aftermath of this, and in the absence of any evidence or attempts at evaluation, the view that hospital is safer became more and more widely accepted.

Tew's challenge is based heavily, but not exclusively, on data from the surveys of 1958 and 1970. She compares mortality rates for babies born in hospitals with those born at home or in general-practitioner units by subdividing births and deaths according to variables known to be associated with different levels of mortality, and uses these data to standardize the overall rates for hospital and other births to allow for the differing numbers of births in each category. She standardizes for single-risk factors, such as the father's social class and the baby's birthweight, and then uses two risk scores, antenatal and labour prediction scores. These are a weighted sum of risk factors identified from analyses of the 1958 survey and then applied to the 1970 data.

In almost every case, mortality rates are higher for hospital births, both in standardized comparisons and in all but the very highest risk categories. Tew interprets this as clear evidence that hospitals are less safe. Is this conclusion valid? The antenatal and labour prediction scores have not been widely validated on other sets of data and their predictive ability is unknown. Studies in other areas that have attempted to use scores of this type to predict the outcome for individuals have not had high degrees of success.

A key question is whether the groups of women giving birth in hospital were directly comparable with the women with the same prediction score who gave birth at home or in a general-practitioner unit. Could there have been other selection factors at work? Tew argues that "no other independent factors have yet been recognised", but it seems unlikely that a woman's obstetric outcome can be predicted unambiguously by the nine items in the antenatal prediction score and the seven in the labour prediction score.

Unfortunately, it is impossible to resolve this dilemma from the available data. Most statisticians and epidemiologists would argue that the way to control for the possibility of selection effects would be to do a randomized trial to compare the safety of birth in different settings and this should have been done before the policies were decided. In the present economic climate, it is most unlikely that such a trial would be undertaken, particularly now that mortality rates are so low that so very many women

would need to take part. Tew argues, on the other hand, that the problems inherent in trials, including the impossibility of double-blinding, possible reluctance to stick to the protocol and Hawthorne effects mean that retrospective analysis of observational data is preferable. Thus the question of relative safety remains unanswered, either way.

*Safer Childbirth?* is useful as a collection of Tew's statistical analyses, but her treatment of history is simplistic. She presents obstetricians as conspiring to produce misleading propaganda to lure hapless women onto their dangerous high-tech territory, and midwives as universally excellent. Obstetricians are certainly not averse either to wielding power or to making unsubstantiated statements, but a conspiracy theory is an unhelpful exaggeration. At the same time, although many admirable midwives have fought to retain and restore their role as the experts in normal pregnancy and delivery, others

have been content to yield responsibility to the medical professions. A reader new to the subject would do better to read the more considered texts cited here by Tew.

The book does not really do justice to Tew's considerable contribution. Although she may overstate the extent to which she has made her case, it is not her fault that the data that would have allowed unanswered questions to be tackled simply do not exist. Further, if she has not fully proved her case, those who disagree with her have not disproved it, or even made a serious attempt to do so. There is no evidence that the fall in perinatal mortality over the past 40 years is a direct result of the change from home to hospital birth, and there is no evidence to support the claim that the safest policy is for all women to give birth in hospital. □

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## Citizens' guide

Ian Stewart

**Journey Through Genius. The Great Theorems of Mathematics.** By William Dunham. Wiley: 1990. Pp. 300. £14.95, \$21.95.

IN THE arts there is a tradition of examining the great masterpieces. Depending on the degree of attention, this tradition ranges from art as a spectator sport to an attempt to recreate what happened in the artist's head. *Journey Through Genius* extends this tradition to mathematics, where "the creative unit is not the novel or symphony, but the theorem". It spans 2,300 years of human creativity, concentrating not on the uses to which the theorems can be put, but on the ideas that led to them and the times in which they were first conceived.

Twelve chapters, twelve great masterpieces. A pity, perhaps, that the word "the" in the subtitle subliminally suggests that there are no other great theorems, for the preface makes it amply clear that these are a selection from a vast cornucopia. A pity also that the most modern theorem is more than 100 years old, and that the only masterpieces after the time of Leonhard Euler (1736) are both the work of Georg Cantor. This is a book about the classics of mathematics, and it should perhaps have said so more clearly; but it is so well written that the criticism is best represented as a plea for a sequel.

A glance at the contents page might suggest a rather clichéd choice of theorems — Pythagoras, the infinitude of primes, the solution of the cubic, the brachistochrone, Cantor's transfinite — but new life is breathed into even the most

familiar of topics. Moreover, there are others, such as Hippocrates' quadrature of the lune, and the Bernoullis' work on harmonic series, that will be new to almost all readers. Indeed, in the popularization of mathematics, the biggest mistake of all is to omit a good thing on the grounds that 'everybody' knows about it. What *everybody* knows about mathematics is non-existent!

The treatment places emphasis on the historical approach, but also on the genesis of ideas. The first chapter, on the quadrature of the lune, is typical. It begins with a historical discussion of the concept of area, going back to ancient Egypt, and leads into the difficulties of assigning areas to shapes bounded by curves. Then Hippocrates' solution of this problem for a lune — a crescent shaped rather like the Moon at its first quarter — is presented at a leisurely pace, making sure that the reader grasps exactly what is involved.

Next, some scraps of historical evidence are pieced together to elucidate a claim of Alexander Aphrodisiensis that Hippocrates had squared (that is, constructed a square of area equal to) the circle — which is now known to be impossible. An ingenious argument involving lunes attached to a regular hexagon shows that the area of a circle can be deduced from the areas of the lunes. Unfortunately, these are not lunes of the type that Hippocrates could handle, so the source of the misunderstanding becomes clear.

Finally, Lindemann's result, obtained in 1882, that  $\pi$  is transcendental, is mentioned, and its application to proving the impossibility of squaring the circle is discussed in more detail. One is left with a well-rounded view of what Hippocrates did, why it was important when he did it, and why it is of interest today to know those things.

Our civilization is founded on mathematics: why do so many of our citizens have no knowledge whatsoever of what that subject involves? *Journey Through Genius* is really aimed at liberal arts students, of a kind that can be found throughout the United States, but which are less common on the other side of the Atlantic. But the book can be recommended to all lively-minded people, whether their interests lie mainly in arts or in science, as a vital cultural experience. □

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## Avian analyses

Robert M. May

**Population Trends in British Breeding Birds.** By John Marchant, Robert Hudson, Steve Carter and Phil Whittington. *Nature Conservancy Council/British Trust for Ornithology*: 1990. Pp. 300. Pbk £12.

POPULATION biologists are often asked what it is that determines the abundance of a particular plant or animal population, and whether the population is increasing or decreasing in response to recent changes. One of the reasons why such apparently simple questions are so hard to answer is that we lack long runs of consistently gathered data on abundances. This is because it is hard to organize (and find money for) the large amount of field work needed to assess population abundance, and even harder to sustain the enterprise over the long sweeps of time — decades or more — that are required for species that take a year or more to attain sexual maturity.

In Britain in the early 1960s, two major and widespread environmental changes became increasingly evident, caused by chemical pesticides and by 'intensification' of agriculture (of which hedgerow removal is the most attention-getting element, but by no means the only one). What are the long-term effects on wildlife? Motivated by this question, and by the fact that birds are more conspicuous than most other animals, the British Trust for Ornithology initiated a year-by-year census of common birds in Britain. Commissioned by the Nature Conservancy Council, this project began with a pilot study in 1961, and started in earnest on farmland in 1962 and in woodland in 1964. This book records the results of the first 27 years of this study.

The Trust solved the massive labour problem of undertaking such a project by making imaginative and disciplined use of its large cadres of amateur members. In the opening chapters of the book, the authors detail how the data were gathered and compiled, allowing for the inevitable

Whitethroat dynamics — showing a marked decline in the late 1960s due to a drought in the Sahel. (Graph taken from *Population Trends in British Breeding Birds*; photo from Ardea.)

problems caused by workers and sampled plots changing somewhat from year to year, and by sampling efficiency varying from person to person. The Trust chose to focus on counting the territories of individual birds during the breeding season, because the constraints of egg-laying means birds are relatively sedentary then. In all, about 200 to 300 local regions or 'plots' were censused each year, with more than 40,000 bird territories mapped in a typical season.

The bulk of the book contains the resulting data, for each of 105 species. Each entry begins with a telegraphic summary of the present population trend (Lapwing: "marked downward trend in the south; probably downward overall", for example) and estimated total size of the breeding population, followed by a graph of year-by-year population change together with a narrative account of the main findings, and concluding with comments on regional variations and trends elsewhere in Europe. Seabirds are excluded, as are those rarities where the nesting population is fewer than 50 pairs. Briefer accounts are provided for an additional 59 species for which the Trust has no census data, so that the book discusses population trends for all terrestrial and freshwater British birds with current breeding populations of more than 50 pairs.

The book is a mine of information. At the British Association meeting in Swansea this year, Peter Lack (of the Trust) used the data it contains to shed light on population trends among the subset of 55 farmland species since the advent of 'intensification'. A typical lowland farm in southern England is likely to contain 30 to 40 of these species, with more than half the individuals in the most abundant five or six species. Broadly speaking, Lack

finds 12 of the 55 species to have increased, 19 to have been relatively unaffected, while 24 have declined. The main decreases, by a factor of around three, are seen in seed eaters (probably because increased herbicide use means fewer of their seeds) and in small hedge species (almost surely owing to destruction or thinning of hedges). Conversely, some larger hedge-and-woodlot species — crow, magpie, wood pigeon — are increasing, probably because they are less persecuted than of yore.

Other changes can be associated with natural events, not least the harsh winter of 1962–63 (which got many of these census-strings off to a low start). Perhaps the most striking example is found among those migratory species which over-winter in the Sahel: sand martin, redstart, sedge warbler, whitethroat and others. These have shown marked decline from the late 1960s onwards, caused by the Sahel drought.

Some changes remain mysterious. As Mike Pienkowski of the Nature Conservancy Council asks in the forward, "why are nuthatches and blackcaps increasing while dunnocks, marsh tits and tree sparrows decrease? Are the decreases in turtle doves, spotted flycatchers and song thrushes linked respectively to usages of herbicides, insecticides and molluscicides?"

This book is a landmark. In a time of growing concern about the biological consequences of climate change, it serves as a guide for long-term studies of other groups of animals and plants, studies we should be starting now. □

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## Exam aid?

Gareth Jones

**Essential Cell Biology.** By C. C. Widnell and K. H. Pfenninger. *Williams and Wilkins*: 1990. Pp. 396. £15.95, \$38.

THE emergence of cell biology as an independent discipline has led to the appearance of two quite outstanding textbooks on the subject that must be familiar to all who have even a passing interest in the subject. Both *Molecular Biology of the Cell* by Alberts *et al.* and *Molecular Cell Biology* by Darnell *et al.* are large books, each of more than 1,000 pages, reflecting the breadth of the field. As both these textbooks, each now published in a second edition, have been universally highly praised, what need can there be for yet another?

If you read the back cover of *Essential Cell Biology*, one or two reasons are provided. The publisher suggests that you don't really want to waste time learning all about plants, and that a compact book with the basics clearly identified is all that is required to pass your exam! This book is aimed at preclinical medical students and biology students who wish to learn cell biology only as part of a foundation course. As such it does a good job, the text concentrating on the principles of cell organization, but with substantial coverage of the functions of specialized cells. There is also a well-written section on techniques in cell biology, plus short model answers to study questions set at the end of most chapters.

In any such book, the necessity to

compress the available knowledge into the briefest possible space demands a high level of skill from the writer. The authors of this one have relied on the help of specialist contributors in executing this task, with mixed success. The chapter on the nucleus, including cell cycle and cell division, is a model of clarity, as is the substantial section on the organelles associated with secretion, endocytosis and intracellular transport. I wished to read more on collagen assembly, elastin and the functions of proteoglycans in a summary of the components and role of the extracellular matrix, and a contribution on the specialized adhesive junctions of cells was disappointing. The casual reader might understand spot desmosomes to be similar to belt desmosomes and that the intercalated disk of cardiac muscle is composed only of spot desmosomes. Much of this confusion could have been avoided if the author had called a spot desmosome a desmosome and a belt desmosome an adherens junction, these being connection sites for intermediate filaments and actin filaments, respectively.

One innovation to be applauded is a chapter on the morphology of specialized cells, in which readers are presented with electron micrographs of various cell types, together with deductive arguments as to their likely identity. Subsequent chapters deal in a straightforward manner with the nature of cell contractility (but why show a figure of frog skeletal muscle, with triads at the Z-disk, to describe a mammalian system based on a pair of triads per sarcomere?), the cell biology of neurons and hormone actions, including second messengers. There is also a chapter on

viral infection which seemed out place; I would have preferred that the space had been used to describe stem cells and the control of cell proliferation. On the whole though, this is a book worth recommending to the target. □

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## Look, no maths

I.F. Croall

**Naturally Intelligent Systems.** By Maureen Caudill and Charles Butler *MIT Press*: 1990. Pp. 296. £14.95, \$19.95.

MANY books have appeared over the past couple of years which provide an overview or introduction to the field of neural networks. Here, Caudill and Butler attempt this task with no use of mathematics, aimed at the general reader. Neural networks, or neural computing, is concerned with the use of very much simplified arrays of processing elements based loosely on biological neurons and their interconnection. Applications are predominantly in fields such as pattern recognition, speech, vision and knowledge processing.

The approach the authors adopt is to provide a brief introduction to the differences between neural networks and conventional computers, then to provide a detailed treatment of a range of different network architectures and examples of applications. The avoidance of even the simplest use of mathematical formulae makes some sections rather ponderous, though a reader with no mathematical background might well have had to skip sections had they been more complex. The lack of mathematics also results in the failure of the book to reveal the relationships between the different classes of networks. There is also little attempt to relate networks to the analogous conventional methods, for example the relationship of multilayer perceptrons to other types of function generators or classifiers.

The episodic nature of the chapters makes the book appear like a series of magazine articles. But despite these caveats, the book is well written and produced. It will provide useful access to the ideas of neural computing. A serious omission is any sort of list of references or further reading suggestions. It is also very much an American view of the subject — the only Europeans mentioned are Hebb and Kohonen. □

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A pair of Asian short-clawed otters with their litter of five cubs. *Operation Otter* by Philip Wayne is the account of he and his wife's attempt to set up an otter reserve, handrearing cubs in order to return them eventually to the wild, thus helping to preserve this fascinating but endangered animal. Published by Chatto and Windus, price is \$17.95 (pbk). □

# New perspectives on freezing and melting

David W. Oxtoby

Over the past decade, new theoretical and experimental techniques have greatly advanced our understanding of the transition between liquid and crystalline phases. Theoretical methods have been developed that view crystallization from the liquid side, in contrast to the traditional lattice-instability theories of melting. Experiments have revealed the ordering of colloidal particles suspended in solution and the disordering of surface layers of crystals, giving direct information about the mechanisms of freezing and melting.

THE crystallization of a liquid is a classic example of the spontaneous appearance of periodic order in a disordered system. What happens on a molecular level to induce long-range oscillations in the average density of an initially uniform liquid? The answer to this question has important practical consequences, in fields ranging from the study of ice formation in clouds to the preparation of rapidly quenched metallic alloys.

Crystallization is a first-order transition—the volume, entropy and other thermodynamic quantities change discontinuously. One of the central characteristics of freezing is that it is highly non-universal: properties such as the location of the freezing point, the changes in thermodynamic variables on freezing, and the symmetry of the stable crystal lattice selected all depend sensitively on the nature of the intermolecular forces. Even for systems as simple as the noble gases there remain open questions about the reasons for the observed stability of particular lattice types under given experimental conditions. Computer simulations do correctly predict that noble gases cooled from the melt will crystallize into a face-centred cubic structure when the atoms are modelled by a simple Lennard-Jones pair potential

$$V_{\text{LJ}}(r) = 4\epsilon \left[ \left( \frac{\sigma}{r} \right)^{12} - \left( \frac{\sigma}{r} \right)^6 \right]$$

where  $\epsilon$  is the potential-well depth and  $\sigma$  is the interatomic separation at which the potential passes through zero. But such a potential also predicts a transition to a hexagonal close-packed lattice at low temperatures which is not observed for the noble gases<sup>1</sup>. To resolve this discrepancy, either the pair potential must be modified or three-body and higher-order terms must be incorporated. The fact that a very small change in pair potential can completely change the symmetry of the crystalline lattice indicates the sensitivity of such phase transitions; simple laws of corresponding states, which frequently hold for the liquid-gas transition, are unlikely to do so for the crystallization of a liquid. In addition, there is the question of how a particular crystalline symmetry is selected, and why metastable crystalline phases are not observed more often than they are.

The freezing of atomic metals illustrates the complexity of the problem. A variety of crystal structures is observed, including face-centred cubic (f.c.c.), body-centred cubic (b.c.c.), hexagonal close-packed (h.c.p.) and more complex structures. The absence of simple trends through the Periodic Table suggests that there exist several structures with very similar free energies, and that *a priori* prediction of the stable structure will be difficult. For example, molten iron crystallizes at atmospheric pressure into a b.c.c. lattice, then converts to an f.c.c. lattice at a slightly lower temperature, and finally returns to a b.c.c. structure at still lower temperatures which persists down to room temperature. Clearly, the two structures have very similar free energies. Gallium presents another interesting example, possessing a liquid phase that is stable over an extraordinarily wide range of temperature: from 2,400 °C to 30 °C (it can be undercooled still further, to –120 °C). When gallium does crystallize, several rather complex stable and metastable phases compete in the

low-pressure region<sup>2</sup>, including one structure with 40 atoms per unit cell. Of course, metals differ from simple atomic systems in that their forces are volume-dependent; this introduces additional subtleties in a complete theory, which lie beyond the scope of this article.

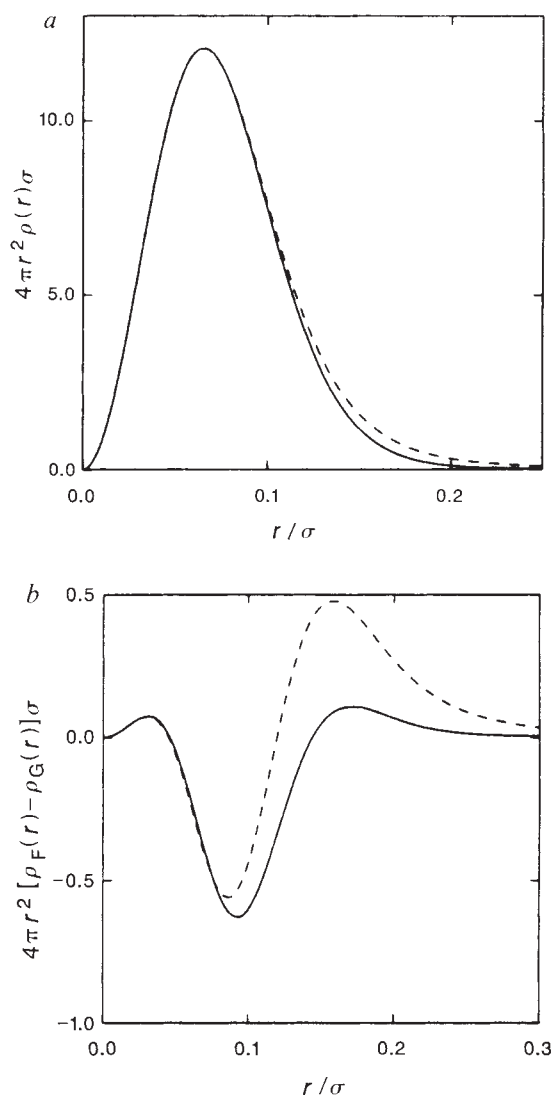


FIG. 1 *a*, The radial density function  $4\pi^2\rho(r)\sigma$  for Lennard-Jones particles at the freezing point measured in three crystal directions from a lattice site: [110] (dashed line), [100] and [111] (solid line). *b*, The deviations of the radial density functions in *a* from that of a Gaussian. Note the greatly expanded vertical scale. From ref. 15.



There is a common misconception that attractive forces between atoms and molecules are responsible for crystallization. Although such forces certainly can play a part in the transition, they are not required for it to occur. For example, a system of hard spheres has purely repulsive interactions—the potential energy is infinite when the spheres are in contact but zero at all larger separations. Yet computer simulations<sup>3,4</sup> reveal that hard-sphere fluids crystallize at sufficiently high density, in a transition that is driven purely by entropy. Other fluids with repulsive potentials crystallize also, such as colloidal particles that interact with purely repulsive Coulomb or screened Coulomb interactions (discussed below). Unlike the gas-liquid transition, which is driven primarily by attractive forces, the liquid-solid transition arises largely from packing considerations and is sensitive to the variation of the repulsive forces with distance.

Three aspects of the liquid-solid transition must be understood before a complete picture emerges. The first concerns the equilibrium properties of the transition: that is, the free energies of liquid and crystalline phases, the changes in thermodynamic properties across the transition, and the microscopic structure of the interface between coexisting phases. The second aspect involves the mechanism by which a new phase first appears: that is, by nucleation in the bulk, or near an impurity, or at the surface. The third aspect is the morphology and rate at which a new phase grows under non-equilibrium conditions. The primary emphasis of this article is on equilibrium freezing and melting, and kinetic aspects of nucleation are considered briefly. Theoretical advances in crystal growth and pattern formation have been reviewed recently<sup>5</sup> and are not considered here.

## Density functional theory

At equilibrium, the temperature and pressure at which a liquid freezes exactly equal the temperature and pressure at which the corresponding solid melts. As a result, theories that attempt to locate the phase transition can start with either phase.

Earlier work has tended to start with the solid phase and look for some instability criterion for melting. Lindemann<sup>6</sup> pointed out that as a crystal is heated, its atoms vibrate about their lattice sites with increasing amplitude. He suggested that once this amplitude reaches a particular fraction (often taken to be 10%) of the nearest-neighbour distance, there will be enough vibration to disrupt the equilibrium crystal lattice. This empirical criterion remains a useful one, and is used to test modern theories of the liquid-solid transition, although no real progress has been made in putting it on firm theoretical grounds. Born<sup>7</sup> proposed that the melting of a crystal occurs when its shear modulus reaches zero. In fact, however, there is no evidence for singular behaviour of the elastic constants of a crystal near melting (on the other hand, such behaviour would be found on approaching a second-order transition). The Born criterion may be useful in estimating the metastability limit of a superheated solid, but not the equilibrium melting point.

In recent years, a new approach to the liquid-solid transition has been developed which begins from the liquid side, using methods that were devised initially to study the equilibrium structure of fluids. These new developments were initiated by Ramakrishnan and Yussouff<sup>8</sup> and were translated into the language of density functional theory by Haymet and Oxtoby<sup>9,10</sup>. The use of density functional methods in statistical mechanics has been reviewed by Evans<sup>11</sup>, and several more specialized reviews<sup>12-14</sup> have summarized their application to the freezing transition. In the density functional approach, the non-uniform average density of the solid (characterized by peaks centred at the lattice sites) is treated as if it were the density of an inhomogeneous liquid. The Helmholtz free energy is a functional of the density, as that in turn is a function of the spatial coordinates. The phase transition is located by comparing the free energy of the 'inhomogeneous liquid' (crystal) with that of the uniform liquid, at the same temperature and chemical potential. This free-energy function may be related directly to the

## Free-energy functionals for non-uniform fluids

The Helmholtz free energy  $F$  of a uniform fluid is a function of its density  $\rho_0$  at a given temperature  $T$ . By contrast, the free energy of a non-uniform fluid is a functional of the density  $\rho(\mathbf{r})$ ; it depends on the global variation of density with position, rather than locally on the density at any given point in space. This functional dependence is indicated with square brackets:

$$F = F[\rho(\mathbf{r})]$$

The central problem in density functional theory is to construct a reasonably accurate Helmholtz free-energy functional  $F[\rho(\mathbf{r})]$ .

For an ideal system (one in which atoms do not interact with one another) the free-energy functional has a particularly simple form:

$$F_{\text{id}}/k_B T = \int d\mathbf{r} \rho(\mathbf{r}) \ln \Lambda^3 \rho(\mathbf{r}) + \text{const}$$

where  $k_B$  is the Boltzmann constant and  $\Lambda$  is the thermal de Broglie wavelength. This is a local free energy, obtained by adding the ideal free-energy densities at all points in space. The exact free energy of an interacting system does not have this simple form. It can be written as

$$F = F_{\text{id}} - \Phi$$

where the correction term  $\Phi$  includes all effects of interactions. Suppose that  $\Phi[\rho]$  is expanded in a functional perturbation expansion about the uniform liquid density  $\rho_0$ . This expansion takes the form

$$\Phi = \Phi_0 + \int d\mathbf{r} C^{(1)}(\mathbf{r}) \{\rho(\mathbf{r}) - \rho_0\} + \int d\mathbf{r} d\mathbf{r}' C^{(2)}(\mathbf{r}, \mathbf{r}') \{\rho(\mathbf{r}) - \rho_0\} \{\rho(\mathbf{r}') - \rho_0\} + \dots$$

where  $C^{(1)}$ ,  $C^{(2)}$ ,  $C^{(3)}$ , ... are the one-, two- and three-body direct correlation functions of the uniform liquid. Because a uniform liquid is isotropic,  $C^{(1)}$  is simply a constant, and  $C^{(2)}(\mathbf{r}, \mathbf{r}') = c(|\mathbf{r} - \mathbf{r}'|)$  depends only on the distance between points  $\mathbf{r}$  and  $\mathbf{r}'$ , not on their absolute positions or relative orientations. The function  $c(r)$  is called the direct correlation function of the liquid.

A key result from liquid-state theory<sup>15</sup> shows that this direct correlation function is related to the liquid structure factor, which can be obtained from X-ray or neutron diffraction experiments or calculated from computer simulation or approximately from first principles. The explicit connection is with the Fourier transform of  $c(r)$ , which is given by

$$\rho_0 c(k) = \rho_0 \int d\mathbf{r} e^{i\mathbf{k} \cdot \mathbf{r}} c(r) = 1 - \frac{1}{S(k)}$$

where  $S(k)$  is the structure factor of the liquid at wave vector  $k$ . In other words, the second functional derivative of the free energy with respect to the density, evaluated for a uniform liquid at density  $\rho_0$ , is related to the structure factor of that liquid. This relation provides a constraint on the form of the free-energy functional that is obeyed by almost all theories for the liquid-solid phase transition. The simplest way to ensure this constraint is to truncate the expansion of  $F[\rho]$  at second order in the density difference<sup>8-10</sup>, which gives the so-called 'perturbation approximation'.

An alternative approach is based on the 'weighted-density approximation', of which several versions exist<sup>18-24</sup>. Typically<sup>20</sup>, these approaches assume that the excess free energy  $\Phi$  depends locally on a weighted density  $\bar{\rho}(\mathbf{r})$

$$-\Phi = \int d\mathbf{r} \rho(\mathbf{r}) \Psi(\bar{\rho}(\mathbf{r}))$$

This weighted density is in turn a non-local functional of the actual density:

$$\bar{\rho}(\mathbf{r}) = \int d\mathbf{r}' w(\mathbf{r} - \mathbf{r}') \rho(\mathbf{r}')$$

The weighting function  $w$  is then chosen so that when the second functional derivative of  $\Phi$  is evaluated, the known direct correlation function is recovered. This approach is non-perturbative in that it includes certain higher terms in the density expansion exactly to all orders; other terms are calculated approximately.

structure factor of the liquid (see box), which, for an atomic liquid, may be measured by neutron or X-ray scattering or calculated accurately using modern methods of liquid-state physics and an assumed pair potential. The goal of density functional theories of freezing is to predict the location and nature of the phase transition entirely from equilibrium properties of the liquid, which are obtained either from experiment or from theory.

It may seem surprising that the highly non-uniform density of a crystal can come out of a theory that begins only with a uniform liquid (together with an assumed crystalline symmetry). But a liquid, although uniform on average, has at any time a local structure about each atom which is reflected in the way it scatters radiation. This local structure becomes long-ranged as the crystal forms. If one writes the free energy as a truncated perturbation expansion about the uniform liquid, there are two locally stable phases that correspond to minima of this free energy: one is the uniform liquid, and the other is a strikingly realistic picture of a crystalline solid. The density distribution of this solid consists of a series of peaks about each lattice site, with a peak amplitude typically several hundred times higher than the average solid or liquid density<sup>15</sup> and a peak shape reasonably close to gaussian (Fig. 1). Thus the zeroth-order description of a harmonic solid emerges naturally in a theory that starts from the liquid side. Even the calculated distortions away from a simple gaussian form make physical sense, with greater probability density along directions in the lattice for which atoms are more distant. For Lennard-Jones particles the gaussian widths at the transition are in quite good agreement with the Lindemann criterion. For hard spheres, the widths are too small relative to experiment, but this may not be surprising given the highly anharmonic nature of hard-sphere systems.

The question naturally arises of whether the success of this truncated perturbation theory is a lucky accident, or whether the higher-order terms that are omitted are truly small. When the best available estimates of the third-order terms are included<sup>16,17</sup> the agreement with computer simulations for hard spheres does in fact get worse. This has stimulated interest in weighted-density approximations (see box)<sup>18-24</sup>, which include certain higher-order terms to all orders. These approximations are successful in describing the gas-liquid transition and the interfaces of liquids with walls and with gases; they generally give good results also for the freezing of hard spheres, although the widths of the peaks in the average atomic density are still too small compared with experiment. Figure 2 shows the results from a weighted-density-approximation calculation of the phase

diagram of the Lennard-Jones fluid<sup>21</sup>. The gas-liquid and liquid-solid coexistence curves shown are in very good agreement with the results of computer simulation. Equally good agreement is found from the truncated perturbation expansion for the liquid-solid transition, although this approach cannot be used for calculating the gas-liquid coexistence curve because the changes in average density between gas and liquid are too large.

It therefore appears that for relatively steep interaction potentials, which cause liquids to crystallize into close-packed lattices, most existing density functional theories give rather accurate predictions of phase diagrams. There are, however, two major theoretical problems that prevent too positive an assessment of the situation. The first arises from a recent density functional calculation for hard spheres<sup>25</sup>, which is based on the fundamental geometries of excluded volume in hard spheres. This calculation gives accurate low-order correlation functions (see box) but fails to give a stable hard-sphere crystal. The second is the failure of existing density functional theories to give the stable body-centred cubic lattices expected for soft repulsive potentials (A. de Kuijper, W. L. Vos, J-L. Barrat, J-P. Hansen and J. A. Schouten, preprint, and B. B. Laird and D. M. Kroll, preprint). For the one-component plasma (a repulsive Coulomb  $1/r$  potential with a uniform background of opposite charge), calculations seem to be extremely sensitive to the accuracy of the input correlation functions, but the current consensus is that thermodynamically stable b.c.c. crystals have not been found<sup>26</sup>. It remains unclear whether these limitations of existing density functional theory can be removed with further modifications, or whether they point toward fundamental inadequacies of liquid-based approaches to crystallization.

### Colloidal crystals

The hard-sphere system considered in the preceding section might seem to be a purely theoretical system that bears no relation to real fluids. In recent years, however, such systems have been prepared and studied experimentally, and have proved a rich source of information about crystallization. The 'hard spheres' are not individual atoms, but nanometre- to micrometre-sized colloidal particles suspended in a background solvent. There are three key factors in preparing such 'sterically stabilized' hard-sphere colloids and studying their crystallization. The first is to ensure that the surface charges on the particles are small, so that long-range Coulomb forces become negligible. The second is to match the refractive index of the colloidal particles closely with that of the background solvent, to reduce attractive dispersion forces between particles and

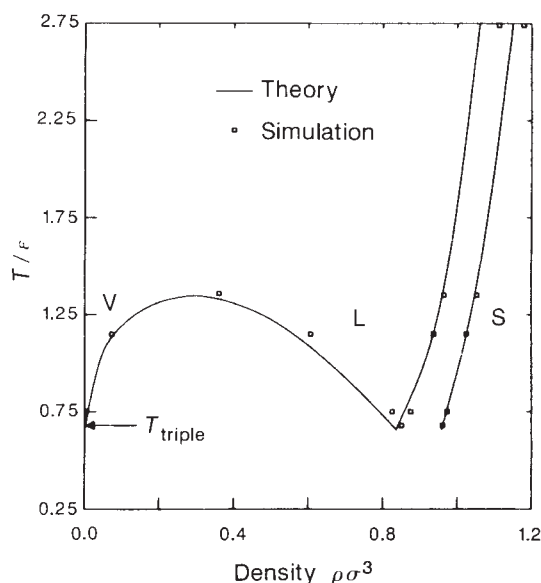


FIG. 2 The temperature-density phase diagram of the Lennard-Jones system calculated using density functional theory (solid line) and found from computer simulation (squares). From ref. 21.



prevent coagulation. The third is to employ nearly monodisperse particles, with a size distribution extending no more than 5–10% about the mean. Colloidal spheres with a polydispersity greater than this will not crystallize. In the most extensive study of these systems<sup>27</sup>, the colloidal spheres consisted of polymethylmethacrylate prepared by emulsion polymerization and stabilized with thin surface layers of poly-12-hydroxystearic acid.

The colloidal fluids in ref. 27 were prepared over a range of colloidal particle volume fractions, and their phase transformations were studied using light scattering. If the volume fraction of the colloid is large enough, phase separation occurs into an upper fluid phase and a lower phase of microcrystals. Because these microcrystals have lattice spacings comparable to the wavelengths of visible light, they cause Bragg diffraction of particular colours of light that depend on their orientation, and so have a sparkling appearance (Fig. 3). (Comparable diffraction from colloidal silica particles is responsible for the play of colours seen in natural and synthetic opals.) The range of volume fractions over which liquid–solid coexistence is seen can be determined experimentally, and the result is in reasonable agreement with the phase diagram of hard spheres obtained from simulations. For large volume fractions, however, crystallization becomes too slow to observe on a laboratory timescale, and a structurally arrested hard-sphere glass forms.

Unlike these sterically stabilized colloids, most colloidal suspensions are charge-stabilized and contain particles with like charges that repel each other through Coulomb interactions screened by ions in the background medium. If such colloids are prepared with reasonably monodisperse charges on the particles and an ion density in the solution low enough for the screening length to be long, they also undergo crystallization at a critical particle concentration. The interactions in such suspensions can be described approximately by a screened Coulomb (Yukawa) potential between point charges

$$V(r) = V_0 \exp(-\kappa r)/r$$

where  $\kappa$  is the inverse screening length, calculable from the Debye–Hückel theory of electrolyte solutions. This approximate potential is not correct if the particle charge becomes too small (or the screening length too short), because then the physical size of the particles becomes important. The phase diagrams of charge-stabilized colloids have been determined from mean-field theory<sup>28</sup>, computer simulation<sup>29</sup> and experiment<sup>30</sup>. The consensus of these studies is that for high charge or long screening length, the fluid crystallizes into a b.c.c. lattice at rather low particle density; if the screening length is reduced sufficiently, crystallization occurs instead into an f.c.c. lattice at a higher particle density.

Charge-stabilized colloidal crystals typically have low particle densities and therefore unusual properties. They are ‘ultraweak solids’, with very small shear moduli compared with normal crystals. As a result, they are easily disturbed by simple agitation of the container, and undergo a series of transitions to increasingly disordered states<sup>31,32</sup>. Theories for this ‘shear melting’ have been proposed<sup>33,34</sup>. Conversely, colloidal fluids that are subjected to shear can undergo shear-induced ordering into string-like structures<sup>35,36</sup>. Presumably, ordinary fluids should also undergo such transitions, but the rates of shear needed would be much higher than for colloidal systems and would not be easily attainable.

Colloidal systems offer an important opportunity to study the dynamics of crystallization. In a simple atomic fluid, the characteristic rate of attachment of particles to a growing interface is of the order of a phonon frequency, whereas in a colloidal crystal it corresponds to the rate of diffusion of a relative large particle over a distance comparable to the lattice spacing in the crystal. This slows growth rates significantly and allows the detailed mechanism of nucleation and growth of crystals to be studied on a convenient timescale<sup>37</sup>. Moreover, the growing crystals can be observed directly with visible light. There seems

to be considerable variation in the growth rates of different types of colloidal crystals. Sterically stabilized (hard-sphere) colloids typically crystallize on timescales of weeks to months, whereas charge-stabilized colloids have timescales of seconds. Smits *et al.*<sup>38</sup> have looked at an intermediate case, in which polymers adsorbed on the surface of the colloidal particles led to a repulsive potential that was softer than hard spheres but steeper than the Yukawa interaction. Such colloids crystallized on intermediate timescales of hours to days. It is unclear whether the differences in timescale are due simply to differences in colloidal particle density at crystallization, or to the changeover from b.c.c. to f.c.c. crystals as the potential becomes steeper.

One final area of recent interest in colloidal transitions is the study of crystallization in monolayers of colloidal particles held between glass sheets<sup>39,40</sup>. Theoretical predictions<sup>41–44</sup> suggest that crystallization in two dimensions may be rather different to that in three dimensions, in that a ‘hexatic’ phase (which has long-range orientational order in the ‘bonds’ connecting nearest-neighbour atoms) may appear between the fluid and crystal phases in the phase diagram. In this picture, a two-dimensional crystal undergoes two second-order phase transitions before reaching the liquid state; these transitions are characterized by the cooperative unbinding of pairs of crystal defects. In refs 39 and 40 the positions of the particles in a two-dimensional colloidal crystal were observed directly with an optical microscope, and the results were consistent with the existence of a hexatic phase, although they could also be accounted for by a first-order transition with a high density of defects. Another study<sup>45</sup> looked at the crossover from two- to three-dimensional behaviour as the number of layers in the crystal was increased.

## Surface melting

At the surface of a crystal, two- and three-dimensional behaviour come together. There has recently been considerable interest in the role of the surface in the solid–liquid transition. It has long been observed that, whereas many liquids can be undercooled quite easily below the thermodynamic freezing transition, the superheating of solids is very difficult to achieve. What is the source of this asymmetry about the equilibrium transition? One suggestion is that liquids crystallize only through the activated process of nucleation<sup>46</sup> in the bulk, or about an impurity or on a wall, whereas solids have free surfaces that permit continuous melting from the outside into the bulk. The idea that a solid may have a thin layer of liquid at its surface is not a new one; Faraday<sup>47</sup> suggested that the slippery nature of ice arises from an outer layer of surface water. Another old idea is that amplitudes of vibration are larger at a free surface, leading to surface melting via the Lindemann criterion. If surface melting does occur, it may have significant consequences besides preventing the superheating of solids; its possible role in frost heave in frozen ground<sup>48</sup> and in electrification of thunderclouds through charge separation in ice–hail collisions<sup>49</sup> have been proposed recently.

The first direct evidence for surface melting came from the scattering of a high-energy (97.5-keV) proton beam from the (110) surface of lead at temperatures below the bulk melting point<sup>50</sup>. The incoming beam was aligned so that if the atoms in the crystal were fixed rigidly at their lattice positions, scattering would occur only from the surface atoms, because the atoms underneath would be shadowed from the incoming beam. At finite temperature, thermal motion leads to scattering from non-surface atoms, but this contribution gives a smooth temperature dependence that can be subtracted. A dramatic increase was observed in the scattering from atoms beneath the surface as the melting point was approached. The data could be fitted by a model in which surface melting begins at 500 K and as many as 20 surface layers melt as the bulk melting point ( $T_m = 600.7$  K) is approached from below. Surface melting does not occur invariably, however: further experiments<sup>51</sup> have shown that the (111) surface does not melt below  $T_m$  and that the ion scattering

FIG. 3 Hard-sphere suspensions, four days after tumbling. The volume fraction of colloidal particles increases from right to left. Note the phase coexistence between liquid and crystal in the samples to the right, and the amorphous glassy phase (blue or green) in the highest-density samples on the left. The larger crystallites in some of the samples were formed by heterogeneous nucleation. From ref. 27.



can be accounted for in this case by thermal motion of crystalline atoms only.

Other experiments have given additional evidence for surface melting. The heat capacities of multilayers of argon and neon adsorbed on graphite reveal strong peaks just below  $T_m$  that can be explained by surface melting<sup>52,53</sup>. Other techniques that have been used include electron diffraction<sup>54</sup> and helium atom<sup>55</sup>, X-ray<sup>56</sup> and neutron<sup>57</sup> scattering. Reference 58 summarizes experimental work to 1987, and ref. 59 includes more recent results. Computer simulations of surfaces of Lennard-Jones crystals<sup>60,61</sup> showed clear signs of surface melting in the outer layers near  $T_m$ , using both structural and dynamical criteria to characterize the state of surface layers. In simulations of aluminium<sup>62</sup>, the close-packed (111) surface showed little evidence of melting, whereas melting of the lower-density (110) surface was established clearly. A simulation of nickel<sup>59</sup> showed that the formation of vacancies and adlayers at lower temperatures permits the disordering corresponding to melting at temperatures close to  $T_m$ .

A simple interpretation of surface melting can be obtained by relating it to the wetting of a solid surface by a liquid. Let  $\gamma_v$  be the surface free energy per unit area of the liquid-vapour interface (the surface tension of the liquid), and  $\gamma_{sv}(\theta)$  and  $\gamma_{sl}(\theta)$  be the solid-vapour and solid-liquid surface free energies per unit area. The latter two depend on  $\theta$ , the orientation of the solid surface. Then if

$$\gamma_{sl}(\theta) + \gamma_{lv} < \gamma_{sv}(\theta)$$

it will be thermodynamically favourable for a thin layer of liquid to interpose itself between solid and vapour—the liquid wets the solid. If, on the other hand,

$$\gamma_{sl}(\theta) + \gamma_{lv} > \gamma_{sv}(\theta)$$

then the liquid will form a surface droplet with a finite contact angle against the solid.

Wetting properties are usually studied for liquids of different chemical composition from the solid on which they are spread. There is no reason, however, why one should not consider the wetting of a solid by its own melt, and it is precisely this phenomenon that is related to surface melting<sup>63</sup>. The layer of liquid will be of less than macroscopic thickness below  $T_m$  (because the solid is the stable bulk phase—there is a cost in free energy to convert solid to liquid) but its thickness will diverge as the bulk melting point is approached. The divergence in liquid thickness is logarithmic for short-ranged forces, but scales as  $(T_m - T)^{-1/3}$  for non-retarded van der Waals forces<sup>64–66</sup>. On the other hand, if a liquid does not wet its crystal, it forms beads on the surface at the triple point (where solid, liquid and vapour coexist). The resulting contact angle has been used to measure the solid-liquid surface free energy of substances such as cadmium and the alkali halides<sup>67</sup>. For lead, there is a clear connection between the estimated relative magnitudes of surface free energies and the melting or non-melting of different surfaces<sup>51</sup>.

A number of theoretical approaches have been advanced to account for the melting of solid surfaces. The phenomenological Landau-Ginzburg theory of phase transitions has been used to place surface transitions that occur near bulk first-order transitions in a very general context<sup>64–66</sup>. A lattice model has been proposed and solved within a mean-field approximation<sup>68</sup>. Most recently, density functional theory has been applied successfully to surface melting (ref. 69 and H. Löwen, R. Ohnesorge and H. Wagner, preprint). This theory shows some of the limitations of Landau-Ginzburg theory for quantitative purposes, and allows surface melting to be related to microscopic properties of the interacting atoms.

If surface melting is the factor that prevents superheating of crystals, it follows that superheating should be possible if the crystal is heated only at its interior, or if it contains no free surfaces. There is some experimental evidence to support this



conclusion. When single crystals of silver were coated with a thin layer of gold to inhibit surface melting, superheating to at least 7.5 K was possible<sup>70</sup>. Small crystallites of lead in an aluminium matrix have been superheated by as much as 67 K above the bulk melting point<sup>71</sup>, although the small size of the crystallites may have affected the extent of the superheating. The theoretical question of what determines the ultimate stability limit of a superheated crystal without free surfaces has attracted recent attention. At high enough temperature, does the crystal change from being metastable (that is, stable to small fluctuations in density while being unstable globally) to being locally unstable? In other words, is there a spinodal in the high-temperature solid? There is good evidence in favour of this hypothesis<sup>72</sup>. One recent suggestion<sup>73,74</sup> is that the stability criterion for a superheated crystal is analogous to that for the glass transition of an undercooled liquid. It is not clear, however, whether the connection to glasses is relevant. Mean-field density functional theory suggests that there is indeed a spinodal for the crystal-to-liquid transition that corresponds to the superheating limit in a mean-field equilibrium theory. On the other hand, such a theory gives no spinodal for the liquid-to-crystal transition<sup>75</sup>, so that ultimately a non-equilibrium conversion to a glass will intervene. If true, this suggests that there is a significant asymmetry in the ultimate fate of a superheated crystal and an undercooled liquid.

### Concluding remarks

Although melting and freezing are inevitably connected at equilibrium by thermodynamics, these two processes differ considerably in their dynamics. Over the past five to ten years the transitions have become accessible to scrutiny with modern techniques: theory, simulation and experiment.

Future progress may rely on closer contacts between physicists or chemists working on theoretical models or on simplified experimental systems, and metallurgists or materials scientists studying problems such as rapid solidification of metal alloys.

It is certainly known that crystallization dynamics can have a significant effect on microstructural properties, but the ways in which such effects arise on an atomic scale are not well understood. Such problems will require the incorporation of quantum-mechanical properties of electrons in metals into statistical mechanical theories of liquids and solids at finite temperature, and will provide an important challenge to theoreticians.

Much of the progress in understanding freezing and melting has involved only equilibrium or quasi-equilibrium aspects: calculating the free energies of coexisting liquids and solids, or measuring experimentally the properties of the equilibrium surface of a crystal. Many of the most challenging problems that remain involve dynamics. What is needed is a better understanding of lifetimes of metastable states, and of atomic-scale effects on nucleation and growth processes for first-order transitions such as freezing and melting. Some information will come from increasingly powerful computer simulation methods, but theoretical work and careful experiments on simple systems such as colloidal suspensions should also provide a wealth of information about dynamics.

Finally, it is inevitable that attention will gradually shift in the future from simpler to more complex systems. For example, many molecular fluids do not undergo a direct liquid-to-crystal transition, but pass through one or more liquid crystalline or plastic crystalline phases, characterized by partial positional or orientational disorder. What factors determine the intervention of these phases, and what are the kinetics of their formation? Complex fluids such as polymers show regions of plastic-crystalline or crystalline order that can have a significant effect on their properties. How do these regions form and how does the microstructure change macroscopic behaviour? These and many other questions remain for the future. □

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ACKNOWLEDGEMENTS. This work was supported by the NSF, in part through the Materials Science Laboratory at the University of Chicago.

# Isotopic evidence from the Ivrea Zone for a hybrid lower crust formed by magmatic underplating

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The large mafic complex overlying mantle peridotites in the Ivrea Zone of northern Italy originated by underplating of mantle-derived magma onto the base of continental crust, and may be a unique outcrop of typical mafic lower crust, sampled elsewhere only by mafic xenoliths. Neodymium and strontium isotope data show that the magmas assimilated large and roughly uniform proportions of crustal material. The resulting hybrid magmas have the same isotopic signature as, and may represent the lower-crustal 'roots' of, upper-crustal Hercynian granitoid intrusions.

THE intrusion of mantle-derived melts into the lower continental crust is believed to be an important mechanism for the growth and chemical modification of the crust. The processes in magma chambers at or near the crust/mantle boundary are believed to include injection of mantle-derived magmas, as well as melting and assimilation of crustal material, fractional or equilibrium crystallization, and tapping of hybrid magmas. They have been labelled AFC (assimilation, fractional crystallization)<sup>1</sup>, MASH (melting assimilation, storage, homogenization)<sup>2</sup> and RTF (refilled, tapped, fractionated)<sup>3</sup>. Such processes would profoundly affect the composition of both mafic and granitoid magmas, and would result in hybrid (crust and mantle) isotopic signatures. Until now, their existence has been inferred from observations on volcanic rocks<sup>2,4</sup> and lower-crustal xenoliths<sup>5-7</sup> rather than from outcrops of (fossil) deep magma chambers themselves. The mafic intrusions of the Ivrea Zone are believed to have been emplaced at the crust/mantle boundary and offer a possibly unique opportunity to study these processes 'in situ'. We focus here on Nd and Sr isotope data from a stratigraphic sequence of gabbroic and ultramafic cumulates overlying the Balmuccia peridotite in the Sesia Valley of northern Italy.

## Geological setting

The Ivrea Verbano area is part of the Hercynian basement of the southern Alps. The SW-NE elongated body is tilted to an almost vertical position and crops out from Ivrea to Locarno. Geophysical evidence<sup>8</sup> and the association of mantle tectonites with plutonic rocks and granulite-facies metasediments has led to the interpretation that this region represents a section through upper mantle and deep crust. The crustal part comprises two formations, the Mafic Complex and the Kinzigite Formation (metapelites, marbles and meta-arenites, interlayered with amphibolites).

The metamorphic grade of the metasediments ranges from amphibolite to granulite facies. The granulites (locally called

stronalites) have lost large amounts of melt, and this created highly restitic parageneses<sup>9,10</sup>, consisting in extreme cases of garnet, sillimanite,  $\pm$ corundum,  $\pm$ spinel and rutile. The mineral assemblages of the Mafic Complex are also consistent with granulite-facies conditions. Hunziker and Zingg<sup>11,12</sup> proposed that both formations have a common metamorphic history. But Rivalenti *et al.*<sup>13</sup>, using field relationships, insist that the Mafic Complex intruded the Kinzigite Formation during or after regional metamorphism and cooled slowly under granulite-facies conditions.

The mafic rocks occur either as large masses, suggesting large magma chambers, or as sills in the metasediments. The particularly thick section exposed in the Sesia Valley consists of a Layered Zone in magmatic contact with the mantle peridotite, overlain by the fairly homogeneous Main Gabbro (MG), which grades upward into the so-called Diorite. The roof of the complex is formed by strongly migmatized amphibolite-facies metasediments.

The Layered Zone has been subdivided<sup>13</sup> into Basal Zone (BZ), Intermediate Zone (IZ) and Upper Zone (UZ) with the following compositional characteristics: BZ, ultramafic cumulates (websterites and peridotites) and gabbros; IZ, also peridotitic and pyroxenitic cumulates and gabbros, but differing from BZ by the coexistence of plagioclase and olivine instead of pyroxene and spinel; UZ, dominantly noritic, with anorthosite layers and rare pyroxene cumulates.

Lenses and major septa of the metasediments with generally sharp contacts are common in the Layered Zone. The highest degrees of depletion (the most restitic mineral assemblages) occur in the metasedimentary xenoliths of the BZ.

## Geochemistry

Table 1 gives stratigraphically ordered, new and published<sup>14,15</sup> element and isotope data for Rb, Sr, Sm and Nd, and information on rock type. Table 2 gives the major-element concentrations and modal abundances for the new samples. Virtually all samples are variably enriched in cumulus phases (pyroxene and plagioclase), with mineral compositions controlled by granulite-facies metamorphic conditions. Other chemical data and attempts to model the major-element and trace-element compositions of the liquids will be published separately. We show the rare-element (REE) and Sr abundances, however, because they illustrate important qualitative features of the evolution of the magmas.

## Age of the intrusion

In previous papers an emplacement age of about 600 Myr was inferred from whole-rock Sm-Nd data for either the entire mafic complex<sup>15</sup> or at least its lower portion, the so-called Lower Layered Group (LLG, corresponding to BZ and IZ in Fig. 2)<sup>14,16</sup>. Figure 1 shows all the available data together with reference isochrons of 600 and 270 Myr. Clearly, these data yield no unique age information, even when the samples are restricted

§ Deceased.



to BZ and IZ. Therefore, we no longer believe that there is any basis for a 600-Myr age of any part of the intrusion. Rather, there are abundant U-Pb determinations on zircons and monazites indicating a major Hercynian event, with ages ranging from 250 to 290 Myr<sup>16-20</sup>. We provisionally adopt an intrusion age of 270 Myr and calculate initial isotopic compositions for that age, the precise value of which is not critical for our purpose.

### Isotope stratigraphy

Figure 2 shows initial  $\epsilon_{\text{Nd}}$  values of the mafic complex in stratigraphic order, not to scale, but with the total thickness of each unit given. Positive  $\epsilon_{\text{Nd}}$  values occur only in the BZ and in the lower part of IZ. Negative values start in the BZ, increase in frequency upwards, and, beginning in the upper IZ, are in a very restricted range ( $\epsilon_{\text{Nd}} = -7.4$  to  $-3.0$ ). Thus, in contrast with conclusions drawn from more limited data<sup>14</sup>, it is now clear that there is no simple stratigraphic break in isotopic composition between the Lower Layered Group (BZ and IZ) and the Main Gabbro, or anywhere else in the section. Another important feature is that in the BZ, the gabbros display a large gap between  $\epsilon_{\text{Nd}}$  values of +6 and -4, and that intermediate values occur only in the ultramafic cumulates. Also shown are average  $\epsilon_{\text{Nd}}$  values and their standard deviations of the underlying mantle peridotite (Balmuccia)<sup>15</sup> and the overlying metapelites (Table 1).

The stratigraphically controlled shift from mantle-like to crustal-like isotopic compositions and the extreme oscillations of isotopic composition in the BZ suggest that crustal contamination was a dominant process. Intermediate isotopic compositions occur only in cumulus peridotites and pyroxenites and we suggest that they represent precipitates (from fresh magma batches) formed early in the contamination process. Supporting evidence comes from metasedimentary xenoliths preserved in the mafic complex. Their highly restitic mineral assemblages indicate that they lost an anatectic melt, which may have acted as a contaminant of the mafic magma. Further evidence for anatexis is provided by the spectacularly migmatized, metapelitic rocks at the top of the complex near Varallo.

Normalized REE and Sr concentrations of each stratigraphic group are shown in Fig. 3. Sr is as incompatible as the light REEs (LREEs) in mantle-derived melts and is placed between Pr and Nd (ref. 21). Most of these patterns have large positive Eu and Sr anomalies characteristic of cumulus feldspar. In addition, there is a general trend of increasing LREE abundances from the BZ to the Diorites, indicating an upward enrichment in incompatible elements in the melts attributed to combined crystallization and contamination.

### The model

A model for the origin of the Ivrea mafic complex must explain the following observations: (1) the erratic sequence of high- $\epsilon_{\text{Nd}}$

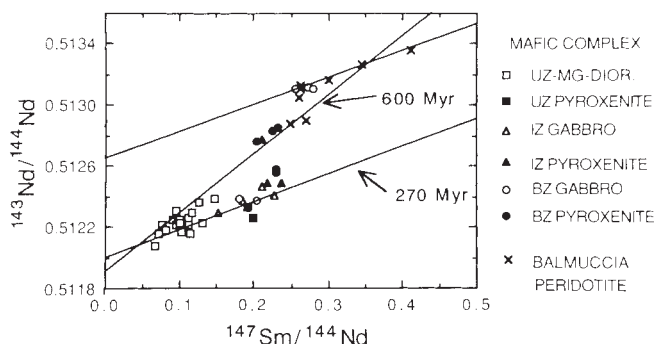


FIG. 1 Sm and Nd data for the Balmuccia Peridotite<sup>15</sup> and the Mafic Complex (Table 1). Filled symbols are for cumulus peridotites and pyroxenites, open symbols for gabbros and diorites. BZ, IZ, UZ and MG represent the stratigraphic units shown in Figs 2 and 3. 'Isochrons' of 600 and 270 Myr are for reference only and have no age significance.

and low- $\epsilon_{\text{Nd}}$  rocks in the lower part of the section; (2) the comparatively uniform and low  $\epsilon_{\text{Nd}}$  values in upper parts of the section; (3) the progressively increasing LREE abundances; (4) the nearly ubiquitous positive Eu and Sr anomalies. We ascribe the first to an AFC<sup>1</sup> stage, and the others to a combination of MASH<sup>2</sup> and modified RTF<sup>3</sup> processes.

**AFC Stage:** Sills of mantle-derived magmas are injected at the mantle/crust boundary, while the crust is at amphibolite-facies temperature. The AFC mechanism operates erratically and produces both uncontaminated melts with positive  $\epsilon_{\text{Nd}}$  and MORB-like REE patterns, and contaminated melts with negative  $\epsilon_{\text{Nd}}$  and LREE-enriched patterns, as well as partially contaminated cumulates.

**MASH-RTF Stage:** A large chamber containing hybrid magma is formed by repeated injection of primary melt, assimilation of crustal material, crystallization and tapping of hybrid magma. The isotopic composition of this magma maintains approximately uniform, negative  $\epsilon_{\text{Nd}}$  values, controlled by a steady-state thermal balance between magma input, anatexis, mixing and crystallization. The magma evolves chemically, however, and becomes more SiO<sub>2</sub> and LREE enriched as a result of continued assimilation, crystallization, replenishment and tapping.

We assume that the vertical stratigraphy corresponds approximately to a time sequence of events. It begins with the injection of sills in the Basal Zone (BZ, stage 1 [AFC stage]), IZ represents the transition to stage 2 (MASH-RTF stage), the formation of a larger magma chamber, and the higher units (UZ, MG and DIO) represent successive cumulates and mixtures of cumulates and melts from the magma chamber formed during stage 2.

During stage 1, some of the sills are thin enough to solidify without inducing partial melting; they therefore retain their mantle isotope characteristics ( $\epsilon_{\text{Nd}} = +6$  to  $+7$ ). Others, because of their volume and the heat supplied by crystallization, induce

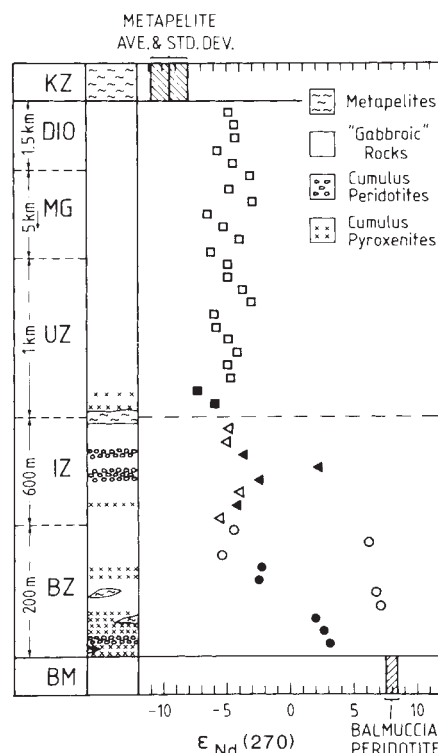


FIG. 2  $\epsilon_{\text{Nd}}$  values 270 Myr ago for the Mafic Complex as a function of stratigraphic position. Symbols as in Fig. 1. Also shown are the means and standard deviations of the Nd compositions for the Balmuccia peridotite (BM)<sup>15</sup> and the metasediments of the Kinzigite Series (KZ, kinzigite and stromatolites; Table 1).  $\epsilon_{\text{Nd}}(270) = \{[(^{143}\text{Nd}/^{144}\text{Nd})_{\text{sample}} / (^{143}\text{Nd}/^{144}\text{Nd})_{\text{bulk Earth}}] - 1\} \times 10^4$  where the superscript 270 refers to a  $^{143}\text{Nd}/^{144}\text{Nd}$  ratio calculated for 270 Myr ago.

initial melting in the metapelites. The amount of anatexis is related to the volume of basalt<sup>22</sup>, and the partial melt separates from the residue only when the melt fraction is sufficiently high<sup>23</sup>. This explains the oscillations of isotopic composition in the BZ and the gap between  $\epsilon_{\text{Nd}} = +6.2$  (uncontaminated gabbros) and  $\epsilon_{\text{Nd}} = -3.0$  (contaminated gabbros). Ultramafic cumulates crystallize early during the contamination process of the liquid and preserve an isotope signature which is intermediate between the initially uncontaminated and the subsequently fully contaminated melt. Overall, this is essentially an AFC process, but one that did not produce the regular changes characteristic of idealized AFC. It is invoked only to explain the erratic isotope variations in the layered series, and it is otherwise not a necessary precursor to stage 2.

During stage 2, the chamber increases in volume and is fed by repeated magma injections. These induce extensive partial melting of the metapelites at the roof, walls and possibly the floor of the chamber. Mantle magmas and crustal melts are mixed and stored in this chamber, and this results in a hybrid liquid undergoing equilibrium crystallization. Consequently, ultramafic cumulates with positive  $\epsilon_{\text{Nd}}$  are no longer formed. The ratio of mantle- and crust-derived melts is controlled thermally (see below), and this explains the narrow isotope range ( $\epsilon_{\text{Nd}} = -3$  to  $-7$ , Fig. 2) and the absence of correlations with stratigraphy and chemical composition throughout the main portion of the complex.

Concurrently, some melt is repeatedly removed from the chamber. Refilling, tapping and crystallization (the RTF process<sup>3</sup>) increases the concentrations of incompatible elements in the chamber by crystallization of feldspar and pyroxene and by replenishment of the magma. This also decouples the behaviour of the major elements, which change much more slowly than the incompatible elements<sup>3,24</sup>. Together with contamination, this explains the upward increase in LREE. The melts removed from the chamber contain significant negative Eu anomalies because they have crystallized the cumulus feldspar remaining in the

chamber. Thus, the bulk residual chamber acquires a complementary positive Eu anomaly. Widespread positive Eu anomalies are also observed in mafic lower-crustal xenoliths<sup>5</sup>.

### Specific processes of the model

**Mixing ratios.** Figure 4 shows Nd plotted against the Sr isotopic composition 270 Myr ago together with calculated AFC<sup>1</sup> and bulk-mixing curves between a mantle-derived melt and two alternative crustal components—a granitic anatectic melt and the bulk metapelitic crust. The AFC and bulk-mixing curves roughly represent model stages 1 and 2, respectively. For all cases, we used a primary magma with 8 p.p.m. Nd,  $\epsilon_{\text{Nd}} = +7$ , 120 p.p.m. Sr and  $^{87}\text{Sr}/^{86}\text{Sr} = 0.703$ . This is consistent with a Sr/Nd ratio (=15) typical of primitive basalts<sup>21</sup> and with the least cumulus-dominated BZ samples (MP1, Q1), which have approximately the major-element compositions appropriate for gabbroic liquids and show the smallest Sr and Eu anomalies (Fig. 3). The isotopic composition of the crustal component ( $\epsilon_{\text{Nd}}(270 \text{ Myr}) = -9.5$ ,  $^{87}\text{Sr}/^{86}\text{Sr} = 0.716$ ) represents the average metasediment from Table 1. The concentrations of the granitic component (35 p.p.m. Nd, 80 p.p.m. Sr) are those calculated by Schnetger<sup>10</sup> for a 40% partial melt (with 70%  $\text{SiO}_2$ ) of Val Strona kinzigites (amphibolite facies), assuming that the average stromalite (granulite facies) represents the residue. (The Nd value has been interpolated from Schnetger's LREE pattern). The concentrations for the bulk-crustal contaminant are the metasediment averages from Table 1 (Nd = 40 p.p.m. and Sr = 220 p.p.m.; for further details, see Fig. 4 caption).

Overall, for the range of  $\epsilon_{\text{Nd}}$  and  $^{87}\text{Sr}/^{86}\text{Sr}$  of interest, the AFC and bulk-mixing curves are similar. The range of isotopic composition of the contaminated gabbros in the BZ and IZ ( $\epsilon_{\text{Nd}} = -4$  to  $-5.6$ ) is reproduced in the AFC models (assuming a ratio of assimilation to crystallization rates of 0.6) by residual

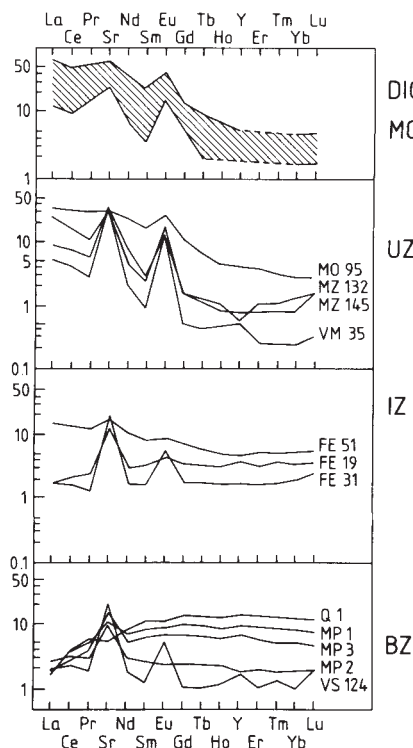


FIG. 3 REE and Sr concentrations normalized to primitive-mantle compositions<sup>21</sup>. The results show a general increase in LREE abundances from the Basal Zone to the Main Gabbro, and nearly ubiquitous positive Sr and Eu anomalies indicating cumulus plagioclase in most samples.

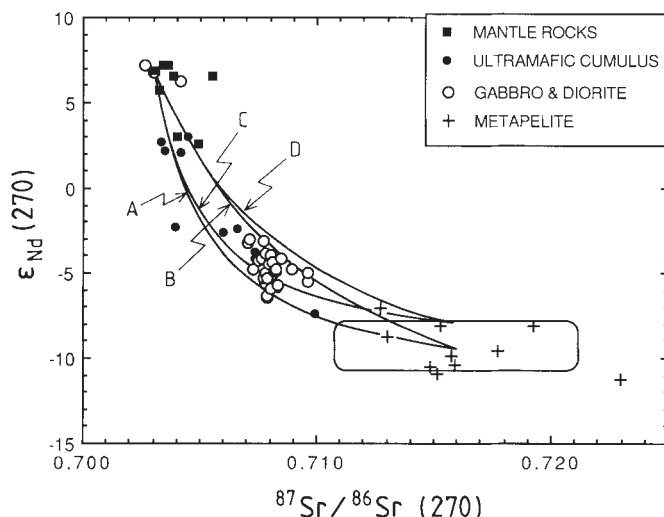


FIG. 4 Initial (270 Myr) Nd and Sr isotopic compositions and mixing curves between mantle and crustal components. The rectangle represents the standard deviation of 10 representative metasediments from the Strona Valley (Table 1). The curves A to D represent mixing models between a primary basaltic melt (120 p.p.m. Sr, 8 p.p.m. Nd) and either an anatectic granitic melt (80 p.p.m. Sr, 35 p.p.m. Nd) as modelled by Schnetger<sup>10</sup> or bulk metapelitic crust (220 p.p.m. Sr, 40 p.p.m. Nd). A, bulk-mixing basalt-granitic melt; B, bulk-mixing basalt-average metasediment; C, AFC-mixing basalt-granitic melt; D, AFC-mixing basalt-average metasediment. A ratio of assimilation to crystallization rates,  $r = 0.6$ , is used in both AFC calculations<sup>1</sup>. The fractionating assemblage consists of 10% olivine, 30% orthopyroxene, 30% clinopyroxene and 30% plagioclase, resulting in bulk  $K_d(\text{Nd}) = 0.087$  and  $K_d(\text{Sr}) = 0.558$ . An alternative fractionating assemblage (20% ol, 30% opx, 40% cpx, 10% plag) yields trajectories in the envelope of the curves shown. Even doubling Sr partitioning to  $K_d = 1.1$  increases the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios only slightly.



TABLE 1 Isotope data

| Sample no. | Rock type | Strat. position | Ref. | Rb (p.p.m.) | Sr (p.p.m.) | Sm (p.p.m.) | Nd (p.p.m.) | $^{87}\text{Sr}/^{86}\text{Sr}$ | $^{143}\text{Nd}/^{144}\text{Nd}$ | $\text{Sr}_i$ (270 Myr) | $\epsilon_{\text{Nd}}$ (270 Myr) |
|------------|-----------|-----------------|------|-------------|-------------|-------------|-------------|---------------------------------|-----------------------------------|-------------------------|----------------------------------|
| GZ7        | px        | BZ              | 14   | 0.29        | 21          | 1.90        | 4.96        | 0.70465                         | 0.51286                           | 0.70450                 | 3.07                             |
| GZ8        | px        | BZ              | 14   | 0.11        | 11          | 0.97        | 2.63        | 0.70347                         | 0.51283                           | 0.70336                 | 2.74                             |
| GZ10       | pd        | BZ              | 14   | 0.04        | 14          | 0.89        | 2.64        | 0.70416                         | 0.51276                           | 0.70413                 | 2.10                             |
| MP 1       | gb        | BZ              | new  | 0.37        | 188         | 3.01        | 7.11        | 0.70265                         | 0.51311                           | 0.70263                 | 7.18                             |
| Q 1        | gb        | BZ              | new  | 0.12        | 91          | 4.35        | 9.65        | 0.70303                         | 0.51312                           | 0.70301                 | 6.79                             |
| Q 6        | px        | BZ              | new  | 0.15        | 14          | 2.43        | 6.41        | 0.70606                         | 0.51256                           | 0.70594                 | -2.58                            |
| Q 10       | px        | BZ              | new  | 0.20        | 56          | 2.46        | 6.46        | 0.70400                         | 0.51258                           | 0.70396                 | -2.28                            |
| IV 41      | gb        | BZ              | 14   | 0.17        | 142         | 0.39        | 1.17        | 0.70964                         | 0.51237                           | 0.70963                 | -5.47                            |
| IV 36      | gb        | BZ              | 14   | 0.44        | 112         | 3.86        | 8.33        | 0.70420                         | 0.51311                           | 0.70416                 | 6.24                             |
| VS 124     | gb        | BZ              | new  | 0.30        | 196         | 0.96        | 3.22        | 0.70804                         | 0.51238                           | 0.70802                 | -4.43                            |
| FE 19      | gb        | IZ              | new  | 0.85        | 272         | 1.23        | 3.25        | 0.70783                         | 0.51241                           | 0.70779                 | -5.60                            |
| FE 21      | px        | IZ              | new  | 0.12        | 11          | 1.10        | 2.80        | 0.70744                         | 0.51249                           | 0.70732                 | -4.20                            |
| FE 31      | gb        | IZ              | new  | 1.41        | 406         | 0.58        | 1.67        | 0.70775                         | 0.51246                           | 0.70771                 | -3.97                            |
| FE 37      | pd        | IZ              | new  | 0.02        | 3           | 0.11        | 0.28        | 0.70667                         | 0.51258                           | 0.70660                 | -2.44                            |
| FE 41      | px        | IZ              | 14   | 0.37        | 246         | 3.48        | 9.91        | 0.70352                         | 0.51278                           | 0.70350                 | 2.22                             |
| FE 49      | px        | IZ              | new  | 0.17        | 12          | 0.63        | 1.72        | 0.70749                         | 0.51249                           | 0.70733                 | -3.72                            |
| FE 51      | gb        | IZ              | new  | 1.07        | 366         | 2.96        | 11.86       | 0.70812                         | 0.51229                           | 0.70809                 | -5.15                            |
| IV 176     | gb        | IZ              | 14   | 0.61        | 377         | 1.04        | 3.43        | 0.70732                         | 0.51237                           | 0.70730                 | -4.82                            |
| GM 90      | px        | UZ              | 14   | 0.20        | 18          | 0.77        | 2.43        | 0.70849                         | 0.51233                           | 0.70837                 | -5.95                            |
| GM 24      | px        | UZ              | new  | 0.16        | 10          | 0.21        | 0.64        | 0.71011                         | 0.51226                           | 0.70994                 | -7.39                            |
| MO 1127    | gb        | UZ              | new  | 0.17        | 116         | 0.39        | 2.17        | 0.70826                         | 0.51224                           | 0.70824                 | -4.75                            |
| MO 95      | gb        | UZ              | new  | 2.66        | 575         | 6.09        | 34.51       | 0.70815                         | 0.51226                           | 0.70810                 | -4.36                            |
| MZ 131     | gb        | UZ              | 14   | 2.68        | 459         | 1.08        | 6.71        | 0.70756                         | 0.51225                           | 0.70750                 | -4.22                            |
| MZ 132     | gb        | UZ              | new  | 1.29        | 535         | 0.87        | 5.57        | 0.70826                         | 0.51221                           | 0.70824                 | -4.87                            |
| MZ 145     | gb        | UZ              | new  | 5.25        | 598         | 1.03        | 9.34        | 0.70794                         | 0.51214                           | 0.70785                 | -5.27                            |
| MAS 6      | gb        | UZ              | 14   | 0.56        | 452         | 2.90        | 13.40       | 0.70807                         | 0.51222                           | 0.70806                 | -5.98                            |
| MAS 5      | gb        | UZ              | 14   | 1.01        | 507         | 0.45        | 2.88        | 0.70776                         | 0.51230                           | 0.70774                 | -3.13                            |
| Kaw 1779   | gb        | UZ              | 15   | 2.11        | 513         | 2.49        | 16.78       | 0.70785                         | 0.51225                           | 0.70781                 | -3.82                            |
| Kaw 1680   | an        | UZ              | 15   | 1.08        | 914         | 0.46        | 3.96        | 0.70779                         | 0.51216                           | 0.70778                 | -4.99                            |
| Kaw 1681   | gb        | UZ              | 15   | 1.37        | 637         | 4.35        | 23.59       | 0.70961                         | 0.51223                           | 0.70959                 | -5.02                            |
| IV 146     | an        | MG              | 14   | 0.66        | 928         | 0.28        | 2.56        | 0.70788                         | 0.51208                           | 0.70787                 | -6.35                            |
| IV 46      | gb        | MG              | 14   | 0.83        | 688         | 5.17        | 27.19       | 0.70802                         | 0.51229                           | 0.70801                 | -3.99                            |
| IV 144     | gb        | MG              | 14   | 2.11        | 539         | 1.52        | 8.26        | 0.70779                         | 0.51221                           | 0.70775                 | -5.36                            |
| MAS 4      | gb        | MG              | 14   | 2.18        | 672         | 1.67        | 8.90        | 0.70790                         | 0.51216                           | 0.70786                 | -6.47                            |
| MAS 3      | gb        | MG              | 14   | 1.71        | 442         | 0.67        | 3.22        | 0.70713                         | 0.51236                           | 0.70709                 | -2.99                            |
| Kaw 1778   | gb        | MG              | 15   | 1.41        | 738         | 0.51        | 3.88        | 0.70823                         | 0.51218                           | 0.70821                 | -4.86                            |
| Kaw 1780   | gb        | MG              | 15   | 1.81        | 355         | 1.17        | 4.76        | 0.70709                         | 0.51239                           | 0.70703                 | -3.18                            |
| MAS 2      | di        | DIO             | 14   | 15.90       | 685         | 5.18        | 27.91       | 0.70819                         | 0.51226                           | 0.70794                 | -4.50                            |
| MAS 1B     | di        | DIO             | 14   | 30.82       | 530         | 3.43        | 20.40       | 0.70894                         | 0.51217                           | 0.70831                 | -5.77                            |
| MAS 1      | di        | DIO             | 14   | 6.72        | 423         | 3.51        | 28.08       | 0.70780                         | 0.51221                           | 0.70763                 | -4.17                            |
| MAS 0      | di        | DIO             | 14   | 30.30       | 428         | 4.29        | 26.07       | 0.70928                         | 0.51225                           | 0.70851                 | -4.21                            |
| Kaw 1785   | di        | DIO             | 15   | 79.00       | 404         | 4.33        | 26.37       | 0.71108                         | 0.51222                           | 0.70896                 | -4.83                            |
| GM Mp      | Stronal.  | IZ/UZ           | new  | ND          | ND          | 8.75        | 36.30       | 0.71628                         | 0.51206                           | ND                      | -9.48                            |
| VS 18      | Kinzig.   | V. Strona       | new  | 132.80      | 207         | 6.57        | 32.90       | 0.72227                         | 0.51209                           | 0.71530                 | -8.17                            |
| VS 14      | Kinzig.   | V. Strona       | new  | 142.00      | 212         | 6.53        | 35.10       | 0.72210                         | 0.51195                           | 0.71485                 | -10.56                           |
| VS 13      | Kinzig.   | V. Strona       | new  | 146.70      | 147         | 6.07        | 31.20       | 0.73008                         | 0.51208                           | 0.71924                 | -8.12                            |
| VS 23      | Kinzig.   | V. Strona       | new  | 89.40       | 256         | 7.28        | 36.40       | 0.71893                         | 0.51194                           | 0.71514                 | -10.93                           |
| VS 10      | Kinzig.   | V. Strona       | new  | 79.70       | 179         | 8.89        | 44.80       | 0.72255                         | 0.51201                           | 0.71771                 | -9.62                            |
| VS 9       | Stronal.  | V. Strona       | new  | 101.80      | 207         | 10.41       | 59.10       | 0.72111                         | 0.51197                           | 0.71576                 | -9.94                            |
| VS 3       | Stronal.  | V. Strona       | new  | 25.00       | 296         | 6.86        | 42.40       | 0.71398                         | 0.51201                           | 0.71306                 | -8.80                            |
| VS 5       | Stronal.  | V. Strona       | new  | 32.50       | 280         | 7.60        | 40.50       | 0.71401                         | 0.51213                           | 0.71275                 | -7.04                            |
| VS 19      | Stronal.  | V. Strona       | new  | 74.80       | 137         | 7.35        | 39.30       | 0.72888                         | 0.51192                           | 0.72295                 | -11.22                           |
| VS 4       | Stronal.  | V. Strona       | new  | 31.00       | 279         | 6.19        | 32.10       | 0.71712                         | 0.51196                           | 0.71592                 | -10.42                           |

Key to abbreviations: px, (cumulus) pyroxenite; pd, (cumulus) peridotite; gb, gabbro; an, anorthosite; di, diorite and monzodiorite; stronal., stronalite (granulite-facies metapelite); kinzig., kinzigite (amphibolite-facies metapelite). BZ, IZ, UZ, MG and DIO refer to Basal Zone, Intermediate Zone, Upper Zone, Main Gabbro and Diorite, respectively. Ref., Reference (by number, or new data); ND, not determined.

melt fractions of 0.74–0.54. Estimated  $\text{SiO}_2$  concentrations of these AFC liquids range from 60 to 67% if the primary liquid is basaltic ( $\text{SiO}_2 = 47\%$ ) and the contaminant granitic ( $\text{SiO}_2 = 70\%$ ).  $\text{SiO}_2$  ranges from 57 to 62% if the contaminant is bulk metasediment with 65%  $\text{SiO}_2$  (assuming a bulk distribution coefficient,  $D(\text{SiO}_2)$  of 0.9).

The bulk-mixing model produces the entire range of  $\epsilon_{\text{Nd}} = -3$  to  $-6.5$  by additions of 27–51% of granitic component, resulting in concentrations of  $\text{SiO}_2$  from 53 to 59%, or 24–47% of bulk metasedimentary crust, resulting in concentrations of  $\text{SiO}_2$  from 51 to 55%. The similarity of the results for the two cases shows that the model is not very sensitive to the specific assumptions for the anatectic component.

The pure AFC process probably yields unrealistically high  $\text{SiO}_2$  values, especially when the contaminant is granitic. The real process is more likely to be intermediate between AFC and bulk mixing, even in the lower portion of the layered series, and the contaminant is likely to be derived from a relatively high degree of melting with  $\text{SiO}_2 = 65\%^{25}$ , favouring  $\text{SiO}_2$  values at the lower end of the calculated range and corresponding to basaltic andesite to andesite liquids. This is consistent with the isotope data, which tend to lie between the extreme assumptions in both models. Even the andesitic melts will have plagioclase, clinopyroxene, eventually olivine, orthopyroxene and, depending on water content, hornblende as liquidus phases<sup>26</sup>, and these are the actual rock-forming minerals found throughout the

TABLE 2 Modal and major-element compositions

| Sample no.                     | MP 1  | Q 1   | Q 6   | Q 10  | VS 124 | FE 19 | FE 21 | FE 31 | FE 37 | FE 49  | FE 51 | GM 24 | MO 1127 | MO 95 | MZ 132 | MZ 145 |
|--------------------------------|-------|-------|-------|-------|--------|-------|-------|-------|-------|--------|-------|-------|---------|-------|--------|--------|
| Rock type                      | gb    | gb    | px    | px    | gb     | gb    | px    | gb    | pd    | px     | gb    | px    | gb      | gb    | gb     | gb     |
| Strat. position                | BZ    | BZ    | BZ    | BZ    | BZ     | IZ    | IZ    | IZ    | IZ    | IZ     | IZ    | UZ    | UZ      | UZ    | UZ     | UZ     |
| Plag                           | 38    | 47    | 0     | tr    | 45     | 50    | 0     | 65    | 0     | 0      | 0     | 3     | 0       | 50    | 65     | 60     |
| Opx                            | 15    | 15    | 35    | 24    | 20     | 25    | 55    | 25    | 0     | 65     | 30    | 83    | 45      | 3     | 25     | 10     |
| Cpx                            | 37    | 35    | 62    | 60    | 30     | 20    | 44    | 8     | 2     | 25     | 9     | 7     | 5       | 15    | 6      | 2      |
| Ol                             | 0     | 0     | 0     | 0     | 0      | 0     | 0     | 0     | 97    | 3      | 0     | 0     | 7       | 0     | 0      | 0      |
| Hbl                            | 6     | tr    | 3     | 3     | 2      | tr    | 0     | 0     | 0     | 5      | 0     | 5     | 25      | 20    | 2      | 20     |
| Gnt                            | 1     | 0     | 0     | tr    | 0      | 0     | 0     | 0     | 0     | 0      | 0     | 0     | tr      | 10    | 0      | 7      |
| Sp, Bi, Ap, Opq                | 5     | 3     | tr    | 13    | 3      | 5     | 1     | 2     | 1     | 2      | 1     | 2     | 3       | 1     | 2      | 1      |
| SiO <sub>2</sub>               | 46.68 | 44.31 | 46.99 | 41.55 | 46.15  | 48.57 | 51.01 | 52.06 | 43.51 | 51.08  | 51.36 | 50.66 | 32.76   | 43.09 | 48.08  | 47.28  |
| TiO <sub>2</sub>               | 1.51  | 2.75  | 0.86  | 1.48  | 0.37   | 1.42  | 0.39  | 0.65  | 0.06  | 0.27   | 1.04  | 0.25  | 5.20    | 2.93  | 0.32   | 0.49   |
| Al <sub>2</sub> O <sub>3</sub> | 13.97 | 15.66 | 6.00  | 14.56 | 21.31  | 17.55 | 4.28  | 21.93 | 0.64  | 3.80   | 20.64 | 4.17  | 7.49    | 20.46 | 26.23  | 26.52  |
| FeO( <i>t</i> )                | 10.98 | 14.06 | 12.69 | 14.36 | 7.65   | 11.07 | 9.20  | 7.38  | 10.25 | 10.54  | 9.00  | 13.17 | 29.48   | 10.10 | 5.69   | 6.83   |
| MnO                            | 0.22  | 0.26  | 0.28  | 0.23  | 0.13   | 0.18  | 0.17  | 0.13  | 0.21  | 0.19   | 0.14  | 0.22  | 0.35    | 0.14  | 0.20   | 0.10   |
| MgO                            | 11.24 | 7.10  | 15.90 | 13.72 | 9.32   | 8.00  | 21.75 | 4.36  | 41.57 | 25.21  | 4.34  | 27.77 | 17.35   | 4.85  | 4.32   | 3.81   |
| CaO                            | 13.75 | 11.67 | 15.28 | 13.27 | 14.23  | 9.64  | 11.40 | 9.14  | 2.54  | 8.25   | 8.58  | 1.64  | 3.87    | 11.42 | 12.17  | 11.11  |
| Na <sub>2</sub> O              | 1.50  | 2.42  | 0.51  | 0.73  | 0.73   | 2.06  | 0.22  | 2.98  | 0.04  | 0.19   | 3.14  | 0.02  | 0.00    | 3.06  | 2.10   | 2.45   |
| K <sub>2</sub> O               | 0.06  | 0.035 | 0.004 | 0.023 | 0.040  | 0.172 | 0.005 | 0.466 | 0.000 | 0.008  | 0.618 | 0.010 | 0.136   | 0.380 | 0.200  | 0.590  |
| P <sub>2</sub> O <sub>5</sub>  | 0.02  | 0.08  | 0.08  | 0.07  | 0.02   | 0.07  | 0.09  | 0.08  | 0.04  | 0.01   | 0.14  | 0.00  | 0.06    | 1.80  | 0.06   | 0.06   |
| LOI*                           | 0.00  | 0.09  | 0.00  | 0.00  | 0.00   | 0.04  | 0.46  | 0.00  | 0.00  | 0.46   | 0.00  | 0.61  | 0.00    | 0.65  | 0.00   | 0.00   |
| Total                          | 99.93 | 98.44 | 98.59 | 99.99 | 99.95  | 98.77 | 98.97 | 99.18 | 98.86 | 100.01 | 99.00 | 98.52 | 96.70   | 98.88 | 99.37  | 99.24  |

Plag, plagioclase; Opx, orthopyroxene; Cpx, clinopyroxene; Ol, olivine; Hbl, hornblende; Gnt, garnet; Sp, spinel; Bi, biotite; Ap, apatite; Opq, opaques.

\* LOI, loss on ignition.

section from the IZ upwards. These compositions also correspond to those of the calc-alkaline (appinitic) dykes, which occur in the country rock of the Ivrea Mafic Complex<sup>27</sup> and may represent tapped liquid from the magma chamber.

We have made no attempt to model the chemistry of the RTF process quantitatively, because too little is known about the evolving chemical compositions of the liquid. Nevertheless, the isotopic compositions modelled by the bulk mixing curves in Fig. 4 are appropriate for the RTF process because the ratio of the continued primary magma and contaminant inputs are postulated to remain constant.

**Single-layer or two-layer magma chamber?** Huppert and Sparks<sup>22</sup> modelled a density-stratified chamber by assuming densities of 2.3 and 2.7 g cm<sup>-3</sup> for the granitic and basaltic magma, respectively. The question arises whether such a stratified magma chamber can be mixed by convection in a sufficiently short time<sup>28</sup>. Examples of hybrid, intermingled magmas, in which inclusions of mafic magma are dispersed throughout large bodies of granitic magmas, have, however, been described<sup>29</sup>. Conceivably, the mafic magma is forcefully injected into, and thereby dispersed throughout, the magma chamber. Alternatively, mixing might be accelerated if the density contrast between the two

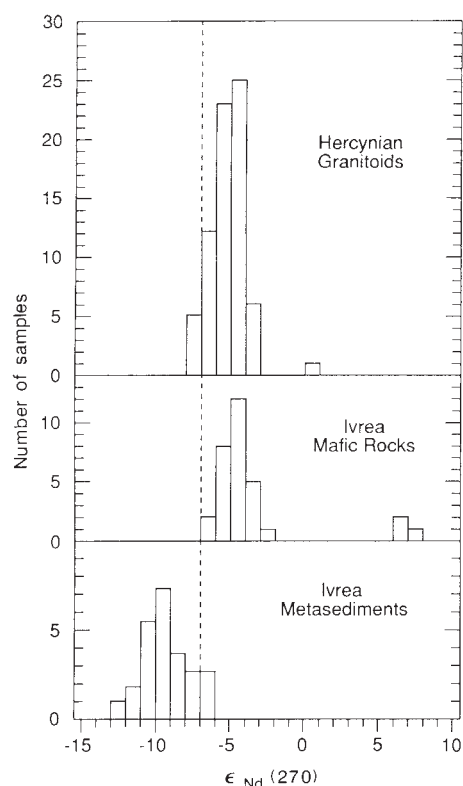


FIG. 5  $\epsilon_{Nd}$  values 270 Myr ago for Ivrea metasediments of the Kinzigite Formation (data from Table 1 and refs 14, 15), the gabbros and diorites from the Mafic Complex (Table 1), and for 72 Hercynian (predominantly I-type) granitoid intrusions and extrusions<sup>4,33-37</sup>.



magmas is reduced, perhaps owing to an elevated volatile content of the hybrid magma. Finally, the presence of metasedimentary septa in the complex indicates that at least part of the granitoid magma was not formed at the roof of the chamber but within it or at its floor. This would considerably enhance the chances for mixing and formation of hybrid magma<sup>30</sup>.

**Heat budget.** The heat budget calculated by Huppert and Sparks<sup>22</sup> yielded a layer of granitoid melt 200 to >1,000 m thick for a 500-m-thick basaltic sill. This is consistent with the mixing ratio found in our calculations (Fig. 4). We suggest an approximately steady-state heat budget for the magma chamber during the MASH-RTF stage. The heat recovered from crystallization of the cumulus phases supplies the heat required to melt the metapelitic crust. The excess heat brought in by each injection of fresh primary magma at 1,250–1,300 °C is predominantly used to heat the anatectic melt from an initial temperature of 800–900 °C<sup>25</sup> to the higher temperature of the hybrid liquid in the chamber. A minor portion is lost by conduction and magma tapping. This heat budget is largely independent of the progressive chemical changes of the magma chamber, and this is the reason why the isotopically determined mixing ratio is not correlated with the chemical evolution of the magma chamber but remains roughly constant.

### Source heterogeneity or *in situ* contamination

We have ascribed all  $\epsilon_{Nd}$  values less than +6 to *in situ* contamination by crustal material. An alternative would be that all isotopic characteristics are inherited from mantle sources, but this would imply that the stratigraphic variations shown in Fig. 2 are coincidental. Two dramatically different mantle sources, and various mixtures between them, would be needed to reproduce the  $\epsilon_{Nd}$  and Sr variations, one with the characteristics of the Balmuccia peridotite, the other with the isotopic composition of the Finero phlogopite peridotite<sup>15</sup>. The fact that the intermediate isotopic compositions between the extremes are found only in cumulus peridotites and pyroxenites would also have to be accidental. Moreover, although the Finero peridotite constitutes a suitable source endmember from an isotope point of view, it is so depleted in Ca and Al, yet so enriched in alkalis that it could act neither as source nor as residue of any of the gabbroic magmas.

Cumming *et al.*<sup>31</sup> argued that the crustal-type Pb isotope signatures of the gabbros were inherited from the mantle. They rejected *in situ* assimilation because they expected to “observe increasing  $\mu$ -values with increasing acidity of the rocks”. ( $\mu = (^{238}U/^{204}Pb)_{present}$  calculated from Pb isotopic composition.) Pin and Sills<sup>14</sup> also argued against “contamination by any likely

crustal contaminants” on the grounds that the Sr/Nd ratios of the metapelites are too high and because “the lack of any obvious relationship between the stratigraphic position and the isotopic composition also argues against a significant amount of contamination”. We disagree with these arguments because: (1) Sr/Nd ratios inferred for anatectic melts formed from the metapelites are consistent with the mixing hyperbolae (Fig. 4); (2) the thermally balanced MASH-RTF process, in contrast with AFC processes, tends to produce hybrid isotopic compositions which do not correlate with elemental concentrations; (3) the AFC-like, non-MASH transition from the Basal Zone to the Upper Zone does show a very strong, overall isotope stratigraphy.

### Significance for upper-crust composition

**Sources of Hercynian granites.** Immediately to the southeast of the Ivrea Zone, there are several granites (such as the Mt Orfano and Baveno granites) that intruded the upper crust about 275 Myr ago<sup>32</sup>, as well as andesitic volcanics of the same age<sup>4</sup>. These have initial  $\epsilon_{Nd} = -4.1$  to  $-5.2$  and  $^{87}Sr/^{86}Sr = 0.707$ – $0.710$ , both of which agree with the values of the Ivrea mafic complex rather than those of the metasediments. Indeed these isotopic compositions are characteristic of most of the Hercynian granitoids of Europe<sup>33–37</sup>. Figure 5 shows a compilation of initial  $\epsilon_{Nd}$  values of 72 Hercynian, predominantly I type (igneous derived) (but including some transitional I–S types [S, sediment-derived]) granitoids from Austria, Czechoslovakia, France, Germany and Italy. These compositions agree well with the Ivrea mafic rocks, and it seems likely that such I-type plutons were derived from lower-crustal parent magma chambers similar to the mafic complex overlying Balmuccia<sup>4,32</sup>.

**Eu anomaly.** The negative Eu anomaly that characterizes the entire upper crust<sup>38</sup> requires a lower-crustal reservoir with a complementary positive anomaly, because mantle-derived melts normally arrive in the crust without a significant Eu anomaly. Granulite-facies metasedimentary rocks and acid igneous rocks generally do not have this property. Conceivably, mafic anorthosites may constitute the necessary reservoir<sup>38</sup>. But, it is doubtful whether the lower crust contains enough anorthosite to balance the upper crust with respect to europium. The Ivrea mafic complex as a whole possesses such an anomaly, which is caused by cumulus plagioclase. In this context, it is important that mafic lower-crustal xenoliths also show this feature<sup>5</sup>. We therefore believe that the Eu deficit of the upper crust may well be balanced by the Eu excess in lower-crustal mafic complexes, which have been formed by processes similar to those seen in the Ivrea Zone. □

Received 19 March; accepted 4 September 1990.

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**ACKNOWLEDGEMENTS.** We mourn the untimely death of the senior author, who completed most of the analytical work and participated in the discussions that led to a reassessment of the probable intrusive age of the Mafic Complex and the consequent reinterpretation of the isotope data. We thank H. Feldmann for help with the Nd and Sr measurements, B. Schneberger and K. H. Wedepohl for representative samples of metapelites from the Strona Valley, and N. T. Arndt, T. C. Liew, S. Moorbath, T. Reischmann, R. Rudnick, P. Stille and G. Wörner for comments and suggestions. This research was supported by the Deutsche Forschungsgemeinschaft, the CNR and the Ministero Pubblica Istruzione.

# Induction of male sterility in plants by a chimaeric ribonuclease gene

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Chimaeric ribonuclease genes that are expressed in the anthers of transformed tobacco and oilseed rape plants were constructed. Chimaeric ribonuclease gene expression within the anther selectively destroys the tapetal cell layer that surrounds the pollen sac, prevents pollen formation, and leads to male sterility. These nuclear male sterility genes should facilitate the production of hybrid seed in various crop plants.

MALE reproductive processes in flowering plants occur in the anther<sup>1</sup>. This organ is composed of several tissues and cell types, contains several thousand anther-specific messenger RNAs<sup>2,3</sup>, and is responsible for producing pollen grains that contain the sperm cells. A specialized anther tissue, the tapetum, plays an important part in pollen formation<sup>1,4-6</sup>. The tapetum surrounds the pollen sac early in anther development, degenerates during the later stages of development, and is not present as an organized tissue in the mature anther<sup>1</sup>. The tapetum produces a number of proteins and other substances that either aid in pollen development or become components of the pollen outer wall<sup>1,4,6</sup>.

Cytoplasmic and nuclear mutations have been identified that prevent normal pollen development and result in male sterility<sup>7</sup>. Many male sterility mutations interfere with tapetal cell differentiation and/or function, indicating that this tissue is essential for the production of functional pollen grains<sup>7</sup>. Male sterility mutations have proved useful for producing hybrids beneficial in increasing crop productivity<sup>7</sup>. Hybrid production, however, has been limited to those plants in which male sterile lines that can be restored to fertility have been identified, and/or those in which mechanical removal of anthers from flowers is both possible and practical<sup>7</sup>. The ability to produce hybrid plants in various crops would be greatly facilitated by the availability of a dominant male sterility gene that could be introduced into plant cells by genetic engineering.

Here we report that the 5' region of a tobacco tapetum-specific gene<sup>8,9</sup> can activate the expression of a  $\beta$ -glucuronidase (GUS) marker gene and two different ribonuclease (RNase) genes within the tapetal cells of transgenic tobacco and oilseed rape plants. Expression of the chimaeric RNase genes selectively destroys the tapetum during anther development, prevents pollen formation, and leads to the production of male sterile plants.

## Tobacco gene expression in anther tapetum

We previously described the identification of two tobacco anther complementary DNA clones, designated as TA29 and TA13<sup>8</sup>. These cDNA clones are 85% similar at the nucleotide level (J. Seurinck, C.M. and R.B.G., unpublished data), and are both complementary to 1.1- and 1.2-kilobase (kb) anther mRNAs that are undetectable in other floral and vegetative organ systems (Fig. 1). *In situ* hybridization studies with anther sections

showed that the TA29 and TA13 mRNAs are both localized within tapetal cells<sup>8</sup>.

DNA gel blot studies indicated that there are less than five TA29- and TA13-like genes in the tobacco genome, and that related genes exist in many other plants, such as tomato, oilseed rape, lettuce and alfalfa (data not shown). We isolated a clone containing the TA29 gene by screening a tobacco genome library with the TA29 cDNA<sup>9</sup>. DNA sequencing studies showed that the TA29 gene does not contain introns, and that it encodes a glycine-rich (20%) protein of relative molecular mass 33,000 ( $M_r$  33K) with potential glycosylation sites<sup>9</sup>. Together, our results indicate that the TA29 gene is expressed specifically in anther tapetal cells, is present in distantly related plant species, and encodes a protein that has properties similar to some plant cell wall proteins<sup>10-12</sup>.

## Control of tapetal-specific expression

Transcription studies with RNAs synthesized in isolated anther and leaf nuclei demonstrated that the TA29 gene is regulated primarily at the transcriptional level (A. Koltunow and R.B.G., unpublished results). To demonstrate that 5' sequences control TA29 gene developmental specificity, we fused the *Escherichia coli* GUS gene<sup>13</sup> with a 1.5-kb TA29 gene upstream fragment (nucleotides -1,477 to +51; ref. 9) containing the start codon,

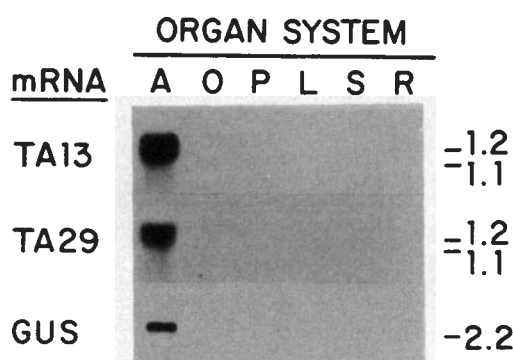


FIG. 1 Organ-specific TA29 and TA29-GUS gene expression patterns. Tobacco anther (A), ovary (O), petal (P), leaf (L), stem (S), and root (R) RNA gel blots were hybridized with either labelled cDNA plasmids (TA13 and TA29) or with a labelled anti-mRNA probe (GUS). Tobacco plants from which vegetative organ systems were collected, as well as flower developmental stages, were described previously<sup>2,3,8,21</sup>. Polysomal poly(A)<sup>+</sup> mRNAs (1  $\mu$ g) from untransformed plants were used for the TA13 and TA29 gel blots. Total RNAs (10  $\mu$ g) from a plant transformed with the chimaeric TA29-GUS gene were used for the GUS gel blot. Autoradiograms were exposed for ~2 h. DNA size markers (kb) to the right.

METHODS. RNAs were isolated as described<sup>2,3,21</sup>. RNA gel blot experiments and labelling of DNA and RNA probes were as described<sup>21,22</sup>. The TA29-GUS gene was introduced into a cointegration vector that contains the bialaphos resistance gene (*bar*) as a selectable marker<sup>18,19</sup>. The TA29-GUS gene was transferred to tobacco (*Nicotiana tabacum* cv. 'SR-1') using standard *Agrobacterium* transformation procedures<sup>18,19,23,24</sup>.

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and then transformed tobacco plants with the chimaeric *TA29-GUS* gene. We generated 13 independent transformants that contained from one to three unrearranged copies of the *TA29-GUS* gene (data not shown). Although the levels varied, the anthers of each transformant contained both *GUS* mRNA and enzyme activity, suggesting that the chimaeric *TA29-GUS* gene was regulated correctly (data not shown).

We hybridized a *GUS* gene probe with different RNAs from one transformant, designated as N102-2, to determine the *TA29-GUS* gene organ specificity. This plant expressed the *TA29-GUS* gene at a level that was about average for all our transformants. *GUS* mRNA was observed only in the anther, and was undetectable in other organ systems (Fig. 1). Experiments with anthers at different developmental stages showed that both *GUS* mRNA and enzyme activity accumulated and decayed in parallel with tapetal cell appearance and disintegration, and were coordinated with changes in endogenous *TA29* mRNA levels (data not shown).

We hybridized *TA29* and *GUS* anti-mRNA probes with adjacent N102-2 anther sections *in situ* to compare the cell-specific expression pattern of the *TA29-GUS* gene with that of the endogenous *TA29* gene. Figure 2a and b shows bright field photographs of anther sections at two developmental stages<sup>8</sup>. Intense hybridization grains were produced by the *GUS* anti-mRNA probe exclusively within the tapetum at stage 2 (Fig. 2g). By contrast, no *GUS* hybridization grains above background were observed at stage 8 when the tapetum had degenerated (Fig. 2h). These hybridization patterns were identical to those produced with the *TA29* anti-mRNA probe (Fig. 2d, e). *GUS* enzyme activity was also detected within the tapetum at stage 2 (Fig. 2i), and was not detectable in other anther tissues. Together, these data show that the chimaeric *TA29-GUS* gene is regulated exactly like the endogenous *TA29* gene, and that the 1.5-kb *TA29* gene 5' fragment contains all the information required to programme the tapetal-specific expression pattern.

### ***TA29-RNase* gene causes male sterility**

We fused the 1.5-kb *TA29* gene regulatory fragment with two different ribonuclease genes to selectively destroy the tapetal cell lineage during anther development. One was a chemically synthesized *Aspergillus oryzae* gene encoding RNase-T1<sup>14</sup>. The other was a natural ribonuclease gene from *Bacillus amyloliquefaciens* called *barnase*<sup>15,16</sup>. Both chimaeric *TA29-RNase* genes were introduced individually into tobacco plants that were either untransformed previously, or that contained a chimaeric *TA29-GUS* gene (N102-2).

We obtained 20 *TA29-RNase T1* and 115 *TA29-barnase* transformants. Sixty per cent of these transformants contained a single *TA29-RNase* gene. The rest had several *TA29-RNase* inserts ranging from two to six copies, depending on the transformant (data not shown). *TA29-RNase T1* and *TA29-barnase* transformants were identical to each other, and to untransformed control plants, with respect to growth rate, height, morphology of vegetative and floral organ systems, time of flowering, and flower coloration pattern. However, 10% of *TA29-RNase T1* transformants (2/20), and 92% of *TA29-barnase* transformants (106/115) failed to shed pollen (Fig. 3). In contrast to mature untransformed anthers (Fig. 3a), anthers on flowers of these plants were shrivelled, greyish-brown in colour, and devoid of pollen grains (Fig. 3b).

The pollenless *TA29-RNase* plants failed to produce fruit capsules and seeds; flowers simply senesced and fell off. By contrast, when these plants were cross-pollinated with pollen from untransformed anthers (Fig. 3a) fruit capsules developed and normal seed set was obtained. These results indicated that the pollenless *TA29-RNase* plants were male sterile, that their pistils were able to recognize and transmit pollen normally, and that female fertility was unaffected. Progeny from cross-pollinated plants with single-copy *TA29-RNase* inserts segregated 1:1 for male sterility and male fertility, and these phenotypes

correlated directly with the presence or absence of a chimaeric *TA29-RNase* gene (data not shown). Together, these data indicate that the presence of either a chimaeric *TA29-RNase T1* gene or a *TA29-barnase* gene leads to the production of male sterile tobacco plants.

We analysed the male sterile anthers of *TA29-RNase T1* plants for the presence of both *RNase T1* and *TA29* mRNAs. In addition, the anthers of male sterile N102-2 plants (Fig. 2) that contained both the *TA29-RNase T1* and *TA29-GUS* genes were analysed for *GUS* enzyme activity. Hybridization of a *TA29* anti-mRNA probe with stage 2 anther sections *in situ* revealed only residual amounts of endogenous *TA29* mRNA (Fig. 2f). This result contrasted with the intense tapetal-specific signals obtained with anthers containing the *TA29-GUS* gene (Fig. 2d, g). Hybridization of adjacent sections with a *RNase T1* anti-mRNA probe failed to produce hybridization grains above background (data not shown). RNA dot blot experiments showed that *RNase T1* mRNA was present in male sterile anthers, but only at a period before the time of maximum *TA29* mRNA accumulation in untransformed plants (stage 1 versus stage 3). In addition, the *RNase T1* mRNA level was at least 100-fold lower than that of either *TA29* mRNA or *GUS* mRNA in anthers containing only the *TA29-GUS* gene (data not shown). In contrast to the high tapetal-specific *GUS* enzyme activity in N102-2 anthers (Fig. 2i), *GUS* enzyme activity was undetectable in male sterile anthers that contained both *TA29-RNase* and *TA29-GUS* genes (data not shown). Together these data show that the male sterility phenotype is associated with a dramatic decrease in tapetal-specific mRNA levels, and that this decrease is correlated with the presence of a chimaeric *TA29-RNase* gene.

### **Selective destruction of the tapetum**

We compared anther development in male sterile tobacco plants containing either the *TA29-RNase T1* or *TA29-barnase* genes to that of untransformed control plants. No differences were observed from stage 1 (0.8 cm flower bud) to stage 12 (open flower) with respect to timing, colour, changes in size and weight, external morphology and filament length. In addition, the male sterile anthers dehisced correctly at flower opening. The single difference between male sterile and male fertile anthers was the apparent absence of pollen grains (Fig. 3a, b).

We prepared transverse tobacco anther sections at each stage of development to compare the tissue differentiation patterns of male sterile and male fertile anthers. Figures 2 and 4 show bright field photographs of stage 2 anthers from male fertile plants (Figs 2a and 4a), and male sterile plants containing a *TA29-RNase* gene (Figs 2c and 4b). Male fertile anthers contained a prominent tapetum (T) surrounding a well-formed pollen sac (PS) that was packed with developing pollen grains (Figs 2a and 4a). By contrast, male sterile anthers lacked a detectable tapetum, and had a collapsed pollen sac without visible microspores or pollen grains (Figs 2c and 4b). All other tissues and cell types were identical in male sterile and male fertile anthers (Fig. 4). Together these data indicate that the presence of a chimaeric *TA29-RNase* gene selectively destroys the tapetum during anther development.

### **Male sterile anthers do not produce pollen**

No pollen grains were observed in any of the 106 male sterile tobacco plants containing a chimaeric *TA29-barnase* gene. By contrast, we obtained a small number of pollen-like structures from dehiscent male sterile tobacco anthers containing the *TA29-RNase T1* gene. These pollen-like structures either failed to germinate or produced abnormal pollen tubes, could not successfully pollinate the pistils of either male sterile or male fertile plants, and were 100-fold less prevalent than pollen grains produced by male fertile anthers (data not shown). We visualized these pollen-like structures in the scanning electron microscope. The abnormal pollen (MS) from male sterile anthers is ~50-fold

smaller in size than normal tobacco pollen grains (WT) (Fig. 5a). Higher magnification of these abnormal pollen grains shows that they do not have a normal exine, lack a groove or sulcus, and are irregular in shape (Fig. 5b). Together, these results indicate that the selective destruction of the tapetum by the expression of the chimaeric *TA29-RNase T1* gene or

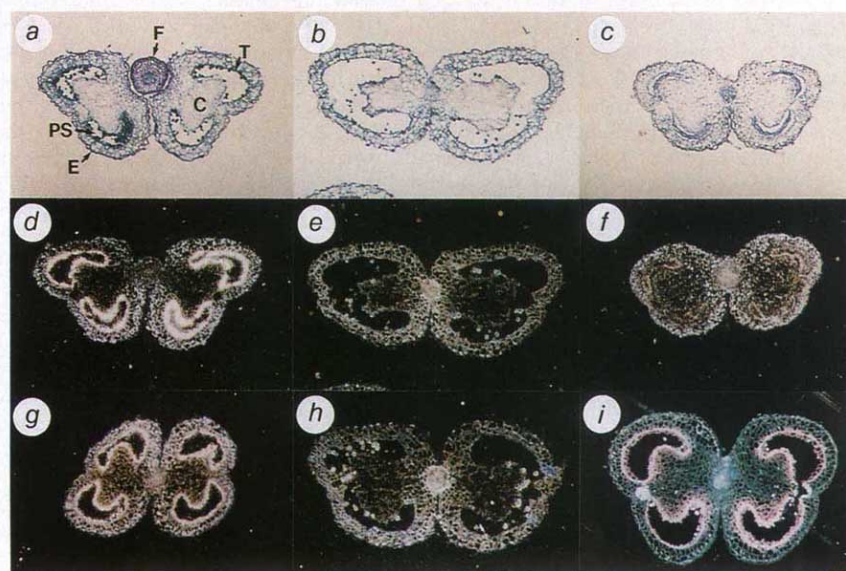
the *TA29-barnase* gene blocks tobacco pollen grain development.

### **TA29-RNase expression in other plants**

We transformed oilseed rape plants (*Brassica napus* cv. 'Drakkar') with the chimaeric *TA29-RNase T1* and *TA29-barnase*

FIG. 2 Localization of *TA29* and *TA29-GUS* gene expression patterns in tobacco anthers. *a* and *b*, Bright field photographs of anthers containing the *TA29-GUS* gene at stage 2 (*a*) and stage 8 (*b*) of flower development<sup>8</sup>. C, E, F, PS and T refer to connective, epidermis, filament, pollen sac and tapetum, respectively. *c*, Bright field photograph of an anther containing the *TA29-RNase T1* gene at flower development stage 2 (ref. 8). *d* and *e*, *In situ* hybridization of a *TA29* anti-mRNA probe with anthers containing the *TA29-GUS* gene at flower development stage 2 (*d*) and stage 8 (*e*). Photographs taken by dark field microscopy. White grains represent regions containing RNA-RNA hybrids. Hybridization grains produced with stage 8 anthers were not detectably greater than those produced with a *TA29* mRNA control probe (data not shown). *f*, *In situ* hybridization of a *TA29* anti-mRNA probe with anthers containing a *TA29-RNase* gene at flower development stage 2 (ref. 8). Anthers from a *TA29-RNase T1* transformant were used for this experiment. Photograph taken by dark field microscopy. White grains along the anther wall represent dark-field light scattering through the stained anther section. Identical results were obtained with a *TA29* mRNA control probe (data not shown). White grains outlining the residual tapetum represent RNA-RNA hybrids, and were only 10-fold greater in density than background grains in adjacent connective tissue. *g* and *h*, *In situ* hybridization of a *GUS* anti-mRNA probe with anthers containing the *TA29-GUS* gene at flower development stage 2 (*g*) and stage 8 (*h*). Sections were taken from the same fixed anthers used for the experiments shown in Fig. 2*d*, *e*. Photographs taken with dark field microscopy. White grains, regions of RNA-RNA hybridization. Grains produced with stage 8 anthers (*h*) represent background hybridization, and were not significantly greater than those produced with a *GUS* mRNA control probe (data not shown). *i*, Localization of *GUS* enzyme activity in stage 2 anthers containing the *TA29-GUS* gene. Pink areas, regions with enzyme activity. Photograph taken with dark field microscopy.

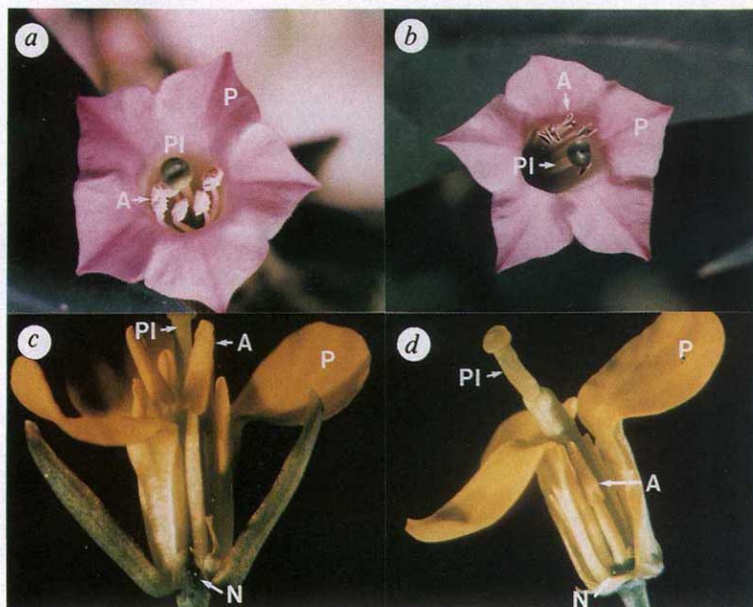
METHODS. Anthers at the relevant stage were collected, and their ends



sliced off with a razor blade to facilitate fixative penetration. Paraffin-embedded anther sections were hybridized *in situ* with single-stranded <sup>35</sup>S-labelled-anti-mRNA and <sup>35</sup>S-labelled mRNA (control) probes exactly as described<sup>21</sup>. Autoradiography and photography techniques used for the hybridized sections were published previously<sup>21,25</sup>. All photographs were taken with the same magnification. *GUS* enzyme activity was localized by incubating unfixed, tipless anthers in 50 mM phosphate buffer, pH 7 containing 1 mM X-Glu (ref. 13) for several hours at 37 °C. After a visible histochemical reaction occurred, the anthers were fixed in glutaraldehyde<sup>21</sup>, embedded in LR-white resin (Polysciences), and sectioned with a glass knife. The *TA29-RNase T1* and *TA29-barnase* genes were recloned into cointegration and binary vectors, respectively<sup>18,26</sup>, and introduced into tobacco plants as outlined in the legend to Fig. 1.

FIG. 3 Male sterile tobacco and oilseed rape flowers. *a* and *b*, Tobacco flowers from untransformed plants (*a*), and plants transformed with *TA29-RNase* gene (*b*). A, P and Pl, anther, petal and pistil, respectively. *c* and *d*, Oilseed rape flowers from untransformed plants (*c*), and plants transformed with a *TA29-RNase* gene (*d*). A, P, Pl and N, anther, petal, pistil and nectar, respectively.

METHODS. The *TA29-RNase T1* and *TA29-barnase* genes were transferred to a binary vector<sup>26</sup> containing the *bar* gene<sup>18</sup>. Oilseed rape hypocotyls (*Brassica napus* cv. 'Drakkar') were transformed with *Agrobacterium* according to the procedure of De Block *et al.*<sup>27</sup> using bialaphos resistance (*bar*) as a selectable marker.





genes to determine whether the tobacco *TA29* gene 5' regulatory region could function in a distantly related plant and lead to male sterility. We obtained 24 *TA29-RNase T1* and 13 *TA29-barnase* transformants that contained one or two intact copies of the relevant *TA29-RNase* gene (data not shown). A male sterile phenotype that cosegregated with the *TA29-RNase* gene was observed in 71% (17/24) and 77% (10/13) of the *TA29-RNase T1* and *TA29-barnase* transformants, respectively. Compared with the flowers of untransformed oilseed rape plants (Fig. 3c), male sterile flowers (Fig. 3d) had slightly smaller petals, contained stamens that did not extend above the petals, and did not contain detectable pollen grains at anther dehiscence. In all other respects the male sterile oilseed rape plants were identical to untransformed controls, including the presence of well-developed nectaries (N) within their flowers (Fig. 3c, d).

We examined the anatomy of male sterile oilseed rape anthers present in 5-mm-long immature flower buds. Figure 4c shows a bright field photograph of a male fertile oilseed rape anther containing a well-formed tapetum (T) surrounding a pollen sac (PS) with developing pollen grains. By contrast, Fig. 4d shows that male sterile oilseed rape anthers containing a chimaeric *TA29-RNase* gene lack a detectable tapetum, and have irregularly shaped pollen sacs that do not contain visible microspores or pollen grains. Scanning electron micrographs of a rare pollen-like body present in only 2 of the 27 dehiscent male sterile anthers (Fig. 5d) showed that this structure was abnormal in size, and lacked a regular exine pattern compared with a normal oilseed rape pollen grain (Fig. 5c). Together, these data show that the tobacco *TA29* gene 5' region functions in oilseed rape anthers, that *TA29-RNase* gene expression selectively

destroys the tapetum, and that the absence of the tapetum leads to male sterile oilseed rape plants.

## Discussion

The experiments presented here show that the *TA29* gene 5' region programmes the expression of *GUS*, *RNase T1* and *barnase* genes specifically to anther tapetal cells, indicating that the *TA29* gene is regulated primarily at the transcriptional level. This finding is consistent with *TA29* gene run-off transcription studies (A. Koltunow and R.B.G., unpublished results), and with earlier population studies that showed that most anther-specific genes are under transcriptional control<sup>3</sup>. Recent studies have shown that only 0.3 kb of 5' sequence is required to programme both the temporal and cell-specific *TA29* gene transcription patterns during anther development (A. Koltunow and R.B.G., unpublished results). These studies suggest that transcriptional events occur during anther development to activate unique gene sets within the tapetum.

The phenotype of plants containing the chimaeric *TA29-RNase* gene strengthens our conclusion that the *TA29* gene is expressed exclusively in the tapetum in both tobacco and oilseed rape plants. If this gene were expressed at other times of the life cycle, the presence of RNase would have disrupted the normal course of vegetative and floral development. Destruction of the tapetum by *TA29-RNase* gene expression does not interfere detectably with anther development, suggesting that the tapetal cell lineage can be eliminated with no effect on subsequent stages of anther development. This result indicates that tapetal cells function autonomously, and that their continued presence is not required for the differentiation and/or function

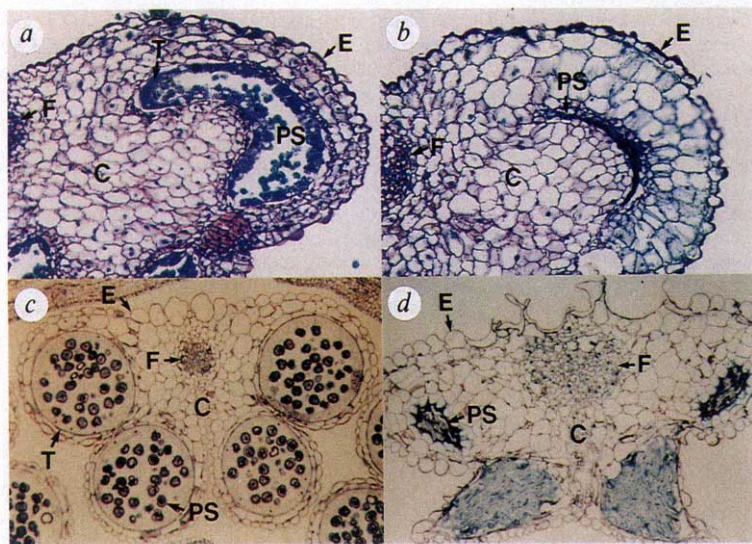
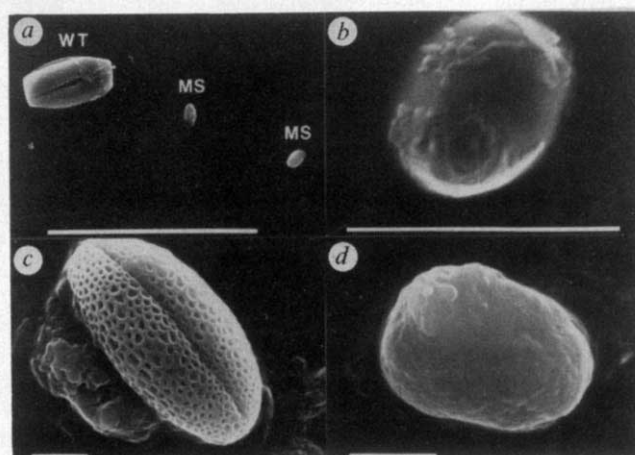


FIG. 4 Tissue abnormalities in male sterile tobacco and oilseed rape anthers. *a* and *b*, Bright field photographs of an untransformed tobacco anther (*a*), and a male sterile anther from a tobacco plant containing a *TA29-RNase* gene (*b*). C, E, F, PS and T, refer to connective, epidermis, filament, pollen sac and tapetum, respectively. *c* and *d*, Bright field photographs of an untransformed oilseed rape anther (*c*), and a male sterile oilseed rape anther from a plant containing a *TA29-RNase* gene (*d*).

**METHODS.** Stage 1 tobacco anthers were fixed and sectioned in a transverse orientation as outlined in the legend to Fig. 3. Oilseed rape anthers were harvested from 2.5-mm flower buds and fixed in glutaraldehyde<sup>21</sup>. Fixed anthers were embedded in LR-white, sliced into 1.5  $\mu$ m transverse sections with a glass knife, and stained with 0.05% toluidine blue.

FIG. 5 Scanning electron micrographs of pollen grains produced by male sterile tobacco and oilseed rape anthers. *a*, Tobacco pollen grains from untransformed anthers (WT), and anthers transformed with a *TA29-RNase* gene (MS). White bar, 100  $\mu$ m. Magnification,  $\times 710$ . *b*, Higher magnification of male sterile tobacco pollen grains shown in (*a*). White bar, 10  $\mu$ m. Magnification factor  $\times 9,150$ . *c* and *d*, Oilseed rape pollen grains from untransformed anthers (*c*) and anthers transformed with a *TA29-RNase* gene (*d*). White bars, 10  $\mu$ m. Magnification factors,  $\times 1,930$  and  $\times 2,980$  for the wild-type and male sterile pollen grains, respectively.

**METHODS.** Pollen grains were collected from dehiscent anthers in open flowers (Fig. 4) and photographed in a scanning electron microscope<sup>28</sup>.



of anther cell types later in development.

The expression of either the *TA29-RNase T1* gene or the *TA29-barnase* gene leads to the production of male sterile plants. These plants are normal in all respects except failure to produce functional pollen. The tapetum is therefore essential for normal pollen development. Expression of both classes of RNase selectively destroyed tapetal cells, presumably by hydrolysing tapetal cell RNAs. An analogous process may occur naturally in the reproductive structures of self-incompatible plants<sup>17</sup>. Barnase seems to be more effective in tobacco than RNase T1. Genetic crosses with male sterile tobacco plants indicated that at least four *TA29-RNase T1* gene copies are required to produce male sterile anthers (C.M. and J.L., unpublished results). By contrast, only one *TA29-barnase* gene copy is required to produce male sterile plants in tobacco, and one copy of either the *TA29-RNase T1* or *TA29-barnase* gene is sufficient to produce male sterile oilseed rape plants. Because the same *TA29* gene 5' fragment was used in both chimaeric

*TA29-RNase* genes, these results suggest that RNase T1 is less active than barnase in tobacco tapetal cells.

The ability of the *TA29-RNase* gene to induce male sterility provides a new strategy for the production of hybrid crop plants. Transferring this dominant male sterility gene to plants such as corn should enable hybrids to be produced without mechanical removal of the anthers. By coupling the chimaeric *TA29-RNase* gene to a dominant herbicide gene (for example, *bar*; refs 18, 19), breeding systems can be devised to select for uniform populations of male sterile plants. In crop plants where fruit is not the harvested product (for example, lettuce, carrot, cabbage), male sterile plants can be crossed with any pollinator line to produce hybrid seeds. By contrast, in other crops such as tomato, wheat, rice and corn it will be necessary to restore full male fertility in the offspring. Antisense RNA technology<sup>20</sup>, and the existence of barstar, a protein inhibitor of barnase<sup>15,16</sup>, should facilitate the development of strategies for male fertility restoration. □

Received 11 July; accepted 24 August 1990.

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ACKNOWLEDGEMENTS. We thank M. De Block, G. Engler, M. Habets and C. Tire for help with the anther sections; K. Cox for teaching us the *in situ* hybridization procedure; P. Stanssens and J. Steyaert for suggestions on RNases and for providing the *RNase T1* gene; R. Hartley for the *barnase* gene; M. Cornelissen and W. Timberlake for critically reading the manuscript; and V. Gossele, H. Van de Wiele, D. De Brouwer and C. Opsomer for technical assistance. Part of this research was supported by the National Science Foundation (R.B.G.).

## A unified model of neutron-star magnetic fields

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**STRONGLY magnetized neutron stars are believed to be at the heart of a number of astrophysical systems, notably pulsars and X-ray binaries. Although the magnetic field is an important determinant in the behaviour of such systems, the origin and stability of the field is the subject of conflicting observational and theoretical evidence. Here I describe a new model of neutron-star magnetic moments, by which the fields are generated as the neutron star is born, and follow the evolution of the field over a Hubble time. With realistic thermal evolution and conductivities, isolated neutron stars will maintain large magnetic fields for more than  $10^{10}$  years. In addition, I show how mass accretion on to neutron stars can reduce the field strength<sup>1,2</sup>. This model of field generation and decay can explain a wide variety of observed systems.**

Newborn neutron stars are thought to have large dipole surface fields, as evidenced by spindown torques on young radio and X-ray pulsars. For a radio pulsar of period  $P$  spinning down in isolation, magnetic-dipole braking models give an estimate of the field at the surface

$$B_s \approx \left( \frac{3Ic^3 P \dot{P}}{8\pi^2 R^6} \right)^{1/2} \approx 1.0 \times 10^{12} (P \dot{P}_{-15})^{1/2} \text{ G} \quad (1)$$

where  $\dot{P}_{-15}$  is the pulsar period derivative in units of  $10^{-15} \text{ s s}^{-1}$ ,

$I$  the moment of inertia,  $R$  the radius and  $c$  the speed of light. For most known radio pulsars, the estimated surface field is  $\sim 10^{12.5} \text{ G}$ , with a dispersion<sup>3</sup> of  $\sim 10^{0.3} \text{ G}$ . The origin of this field is a subject of long-standing debate; a popular scenario involves flux freezing and amplification of interior fields of the progenitor object, which leaves the existence and magnitude of the characteristic value unexplained. Alternatively, it has been proposed that magnetic fields can be generated in young stars by thermomagnetic effects<sup>4</sup>.

The torque appears to decay on a timescale of several million years, leading to a rapid disappearance of the pulsars from the radio sky<sup>3,5</sup>. Recent observational and theoretical work has, however, clouded this picture appreciably. The binary and millisecond radio pulsars, in particular, make it clear that the fields of some neutron stars can be both a factor of  $\sim 10^4$  lower than the characteristic value and remain stable for  $\geq 10^9 \text{ yr}$  (ref. 6). Conversely, some  $\gamma$ -ray burst sources, believed to be old neutron stars, have been shown to have high surface fields of  $\geq 10^{12} \text{ G}$  (ref. 7); in addition, theoretical arguments<sup>8,9</sup> imply that it is difficult to reduce the magnetic flux threading much of the neutron-star interior.

Here I present a simple picture of the evolution of the magnetic field that explains the above behaviour. I assume that the field is generated after the birth of the neutron star during the initial cooling phase, either by thermomagnetic effects in the solid crust<sup>4</sup> or by dynamo processes in the still-liquid envelope above the solid interior. Thermomagnetic generation is most effective where the temperature gradient is steepest, that is, at the lowest solid densities. Similarly, dynamo-generated flux will be frozen into the star at the base of the liquid sea. Thus the yield stress



of the lattice at the melt surface provides a maximum value for the local dipolar field that can be frozen into this outer crust. I assume that the (unprescribed) mechanism of field generation is effective, saturating at this critical value; higher fluxes will break the Coulomb lattice and escape from the star. Balancing the magnetic stress with the yield stress of the lattice determined from the critical strain angle  $\varepsilon$  and the shear modulus  $\mu$ , the critical field is

$$B_c = (8\pi\varepsilon\mu)^{1/2} = 3.7 \times 10^{13} \varepsilon_{-2}^{1/2} Z A^{-2/3} \rho_{11}^{2/3} G \quad (2)$$

where  $\varepsilon_{-2}$  is in units of  $10^{-2}$  radians,  $\rho_{11}$  is the density in units of  $10^{11} \text{ g cm}^{-3}$ ,  $Z$  is atomic number and  $A$  is the mass number of the equilibrium lattice nuclei. As the star cools, the melt surface moves out to lower densities; when the underlying field dominates resistance to motions of the melt surface at  $\rho \approx 10^7 \text{ g cm}^{-3}$ , the addition of flux to the solid will cease.

I have taken results of a cooling calculation for a model star of mass  $1.4 M_\odot$  and radius  $R_*$  (ref. 10) as typical of the thermal evolution of neutron stars. Using the analytic results of ref. 11, I obtain the temperature behaviour through the solid crust between the core (isothermal below neutron drip) and the melt surface at  $T_m = Z^2 e^2 / k \Gamma_m (4\pi\rho/3m_p A)^{1/3}$  where  $e$  is the electron charge,  $k$  the Boltzmann constant, and  $m_p$  the mass of the proton. Here the plasma parameter  $\Gamma_m$  is  $\sim 160$ , and the local composition  $Z$  and  $A$  are determined from the results of ref. 12 for  $\rho \leq 2 \times 10^{11} \text{ g cm}^{-3}$ , and from the calculations of Buchler and Barkat at higher densities<sup>13</sup>. At late times I include interior vortex-creep heating<sup>14</sup> which generates a surface temperature

$$T_s \approx 2.9 \times 10^4 (I_{\text{cr},43} \omega_{c,-2} \dot{P}_{-15} P^{-2})^{1/4} \text{ K},$$

where  $I_{\text{cr},43}$  and  $\omega_{c,-2}$  are the crustal moment of inertia and the critical slip velocity in units of  $10^{43} \text{ g cm}^2$  and  $10^{-2} \text{ rad s}^{-1}$ , respectively.

The field in the solid crust will undergo slow ohmic diffusion<sup>15</sup>. I am interested only in the dipole component of the anchored field, and so follow ref. 9 by taking the field decay in the solid, where internal motions are negligible, to be described by

$$\frac{\partial g}{\partial t} = \frac{c^2}{4\pi R_*^2 \sigma(r, t)} \left( \frac{\partial^2 g}{\partial r^2} - \frac{2}{r^2} g \right) \quad (3)$$

where  $\sigma$  is the conductivity. Here  $g(r, t) = r A_\phi / \sin(\theta)$  is the time-dependent one-dimensional 'vector potential', depending only on radius, which completely describes the dipole-field distribution. The perpendicular magnetic moment is given by  $m_z = 3/8\pi \int_{R_*} B_z d^3r = g(R_*) R_* = B_s / R_*^2$ .

There are two important contributions to the resistivity in the crust of neutron stars. The first is scattering of electrons by

phonons in the Coulomb lattice. I calculate this conductivity at each depth and temperature by interpolating the numerical results of ref. 16, reduced by a factor of about three to bring them into agreement with the more limited calculations of ref. 17. This will be the dominant source of resistivity at high temperatures and low densities. At late times, however, scattering by a lattice impurity fraction  $\chi$  will dominate, leading to a conductivity of  $\sigma_{\text{imp}} = 2 \times 10^{23} (Z\rho_{11}/A)^{1/3} Z / (\chi \Delta Z^2) \text{ s}^{-1}$ , independent of the temperature. Simple estimates<sup>18</sup> suggest that  $\chi \Delta Z^2 \approx 10^{-3}$  for crustal matter; I adopt this value as fiducial. These conductivities add harmonically in the crust. For  $\rho \geq 10^{14} \text{ g cm}^{-3}$  little diffusion occurs; I take the conductivity to be the core value determined by the superconducting protons  $\sigma_c = 1.5 \times 10^{36} \rho_{11}^{3/2} / T_9^2 \text{ s}^{-1}$  where  $T_9$  is the core temperature in units of  $10^9 \text{ K}$ .

I constructed a model star by integrating the Oppenheimer-Volkoff equation with the adopted equation of state to get  $\rho(z)$ . With  $T(t)$  and  $\sigma(t)$  as above I evolved the model, following the growth and ohmic diffusion of  $g$ , solving equation (3) by a forward-backwards symmetric Crank-Nicholson scheme. During the growth phase the field at the melt was held at the saturation value  $B_c$ ; after growth ceases the boundary condition excludes surface currents. At all times the field was held to be less than the local  $B_c$  throughout the star; excess flux was presumed to break the lattice, advect to the surface and disappear. Details of the calculations will appear elsewhere (manuscript in preparation).

The results are shown by the heavy line in Fig. 1. The calculation starts 0.2 yr after the birth of the neutron star, when the melt surface has reached neutron drip and the lattice is fully formed. The field grows quite rapidly, reaching  $10^{12} \text{ G}$  after only a few years. The simple prescription for the initial conditions produces the typical field quite well. The behaviour is not very sensitive to the age at which growth starts,  $t_0$ —maximum fields are  $\sim 10^{12} \text{ G}$  for  $t_0$  as large as 10 yr. The dependence on the critical strain angle ( $\varepsilon^{1/2}$ ), stellar radius and moment of inertia are, however, adequate to explain the observed range of  $B$  values.

Growth ceases at  $\sim 10^6 \text{ yr}$ , and the field decays ohmically in the outermost crust on a timescale of  $\sim 10^6 \text{ yr}$ . This partial screening of the interior flux gives a  $\sim 30\%$  decrease in  $B_s$ . Hereafter the star is sufficiently cold that flux is well frozen into the crust. Only a slight decay on a timescale  $\geq 10^{10} \text{ yr}$  is apparent owing to the impurity scattering resistivity. The adopted model loads the outer crust with the maximum allowed field; other initial conditions should produce configurations with currents held deeper in the star and longer ohmic decay times. Thus the

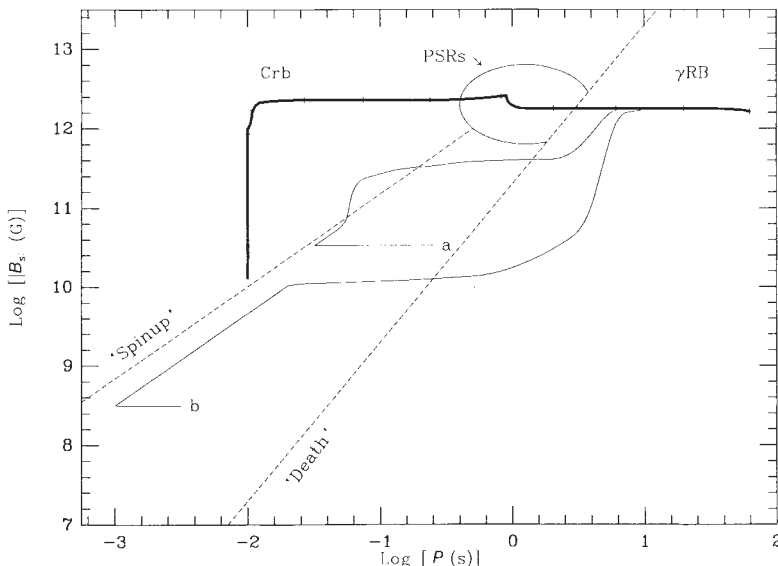


FIG. 1 Neutron-star dipole field plotted against spin period. The heavy full line gives the track of an isolated star evolving from  $P=10 \text{ ms}$  at  $t_0=0.2 \text{ yr}$ . Tick marks indicate the age of the star, spaced at 1 yr, 10 yr, ... The recycled pulsars are found between the dashed 'death' and 'spin-up' lines (shown for an Eddington accretion rate). Thin solid lines sketch the evolution of accreting systems; objects can reappear as radio pulsars on the horizontal line segments at the ends of the tracks. The observed locations of typical pulsars (PSRs), Crab-like objects (Crb), and  $\gamma$ -ray burst sources ( $\gamma$ RB) are also indicated.

conclusion is quite robust—with realistic temperatures and conductivities an initial magnetic field can decay ohmically by only a modest factor.

Figure 1 shows the evolution in the  $P$ - $B$  diagram, when the spin period is 10 ms at  $t_0$  (full line). The star moves rapidly to the region in which young pulsars, such as Crab and Vela are found. As the track evolves to longer periods there is still a modest increase in  $\dot{P}$  for the young objects, corresponding to 'braking indices' less than three, as observed for several such pulsars<sup>19</sup>. After joining the bulk of the observed pulsars, at  $P \approx 1$  s,  $B \approx 10^{12}$ , the  $B_s$  drops slightly and crosses the so-called 'death line' at  $B_{12}/P^2 \approx 0.2$ , beyond which the magnetospheric potential cannot support pulsar emission. This decrease can help explain the observed torque decay of radio pulsars, possibly abetted by additional resistivity in the surface layers and alignment of the magnetic and spin axes. After disappearing from the radio sky, the pulsars continue to spin down, reaching  $P \approx 30$  s in a Hubble time. The parameters in this pulsar graveyard agree well with the limited observations of  $\gamma$ -ray-burst magnetic fields<sup>7</sup> and spin periods (such as GRB030579).

It has been noted<sup>1,20</sup> that all neutron stars with anomalously low magnetic moments are in mass-transfer binaries or resided in such systems in the past. Contact binaries produce the 'recycled' binary, millisecond and globular-cluster pulsars. There is even evidence that the magnetic moments of these objects are correlated with the duration of the mass-accretion phase or the total amount of matter accreted<sup>1,21,22</sup>. In the past, these discussions appealed to either an inverse battery effect<sup>23</sup> or an accretion-induced collapse origin of the resultant pulsars, both of questionable efficacy.

Having calculated the model's long-lived residual field, its subsequent modification under mass accretion can be described. There are two principal effects. The energy released during accretion will reheat the outer layers of the neutron star to  $T_h \approx (GM_*\dot{M}/4\pi R_*^3\sigma_B)^{1/4}$  (where  $G$  is the gravitational constant,  $\sigma_B$  the Stephen-Boltzmann constant,  $M_*$  the mass of the star and  $\dot{M}$  its accretion rate), lowering the conductivity and hastening ohmic decay. Far more important, however, is the compression of the outer layers of the star by the accreted overburden and the inward advection of horizontal components of the crustal magnetic field. Matter flowing onto the polar cap is channelled by the magnetic field, building up until the static pressure is comparable to  $B^2/8\pi$ . At this point material will begin to move sideways on a free-flow timescale. For strong fields the magnetic pole restricts the flow until densities of  $\sim 10^6$  g cm<sup>-3</sup>, where the field is relatively well coupled to the matter. Thus the spreading material will decrease the density of field lines at the magnetic pole, carrying them towards the equator, where they will be advected below the surface. These flux lines are trapped in the underlying crust and screened by the diamagnetic accreted material. As the field at the polar cap decreases, the matter will tend to leak out closer to the surface, at lower densities. For surface fields of  $\leq 10^9$  G the accretion column will be disrupted at 1–10 g cm<sup>-3</sup> where the ohmic decay and fluid interchange timescales will be shorter than the flow timescale. Thus material will no longer drag the magnetic flux lines beneath the surface, although it will continue to advect the current-carrying layers deeper into the star. The resultant asymptotic value of the field will depend on the total amount of mass accreted and, through the surface reheating and flow timescale, on the accretion rate. Calculation of the field motion through the crust for particular accretion histories can be used to relate the bright, rare X-ray binaries to their radio-pulsar descendants.

Figure 1 shows tracks of the resultant field and spin evolution. The accreted matter adds angular momentum<sup>24</sup>, moving the star to smaller  $P$ . Systems with high  $\dot{M}$  and short accretion lifetimes (wide low-mass X-ray binaries and common-envelope systems) will follow tracks such as track a. Initially, high transfer rates will spin up the neutron star, producing a short-period X-ray

pulsar. The total mass accepted from the transfer is  $10^{-5}$ – $10^{-3} M_\odot$ , however, causing only modest field reduction and producing systems such as PSR0820+02, PSR0655+64 and PSR1913+16. Binaries with longer-lived accretion phases and lower rates of mass transfer will result in neutron stars with masses substantially increased above their birth mass of  $\sim 1.4 M_\odot$ , and fields decreased to near the asymptotic value above (track b). The result should be recycled objects such as PSR1953+10 and PSR1620+21 near the 'spin-up line', gradually slowing due to dipole braking by the residual magnetic field. For both types of pulsars the currents and fields have been advected to the high-conductivity interior of the neutron star, precluding additional decay of  $B_s$ , a further virtue of this model.

The specific predictions that these calculations give for the resultant mass and spin properties should allow stringent observational tests and further refinement of the models.  $\square$

Received 30 April; accepted 28 August 1990.

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ACKNOWLEDGEMENTS. I thank L. Hernquist and J. Applegate for advice and discussions. This work was supported by the NSF and Corning Glass Works.

## Global increase of atmospheric molecular hydrogen

M. A. K. Khalil & R. A. Rasmussen

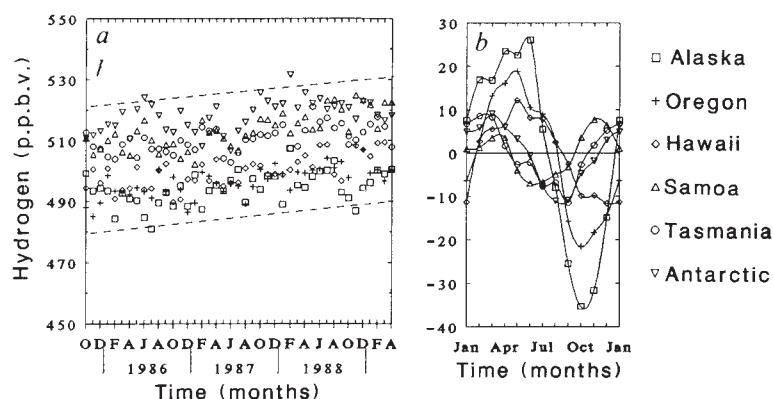
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**It is now well known that levels of atmospheric CO<sub>2</sub>, CH<sub>4</sub>, N<sub>2</sub>O and CO are increasing at substantial rates as a result of human activities. Here we report that levels of another trace gas, molecular hydrogen, are also increasing. Our systematic measurements between 1985 and 1989 show that the concentration of H<sub>2</sub> increased at an average rate of  $3.2 \pm 0.5$  parts per 10<sup>9</sup> by volume per year (a relative increase of  $0.6 \pm 0.1\%$  yr<sup>-1</sup>). These increases originate from anthropogenic sources. Higher levels of hydrogen will add more water vapour to the stratosphere, where it can affect stratospheric ozone.**

During the past decade we have taken weekly air samples, in triplicate, at sites ranging in latitude from the Arctic to the South Pole (Pt Barrow, Alaska, 71.5° N; Cape Meares, Oregon, 45.5° N; Cape Kumukahi and Mauna Loa, Hawaii, 19.3° N; Cape Matatula, Samoa, 14.1° S; Cape Grim, Tasmania, 42° S; South Pole and recently at Palmer Station, Antarctica, 71.4° S). Air was collected using specially designed pumps and stainless steel internally electropolished 0.8-l flasks<sup>1</sup>. Samples taken at most sites were analysed within a few weeks of collection but laboratory studies show that most gases, including H<sub>2</sub>,



FIG. 1 *a*, Monthly average concentrations of  $H_2$  at six sea-level sites in the polar regions, middle latitudes and the tropics of both hemispheres. Seasonal cycles have been subtracted. *b*, The average seasonal cycles at the six sites. The measured monthly average concentration is the sum of the average cycles in *b* and the 'de-seasonalized' concentrations in *a*.



are preserved in our containers for much longer. Hydrogen concentrations were measured with a gas chromatograph (column: molecular sieve 5A,  $3' \times \frac{1}{4}''$ ) and a  $HgO$  gas-reduction detector manufactured by Trace Analytical Inc. The precision of the measurements is  $\pm 0.5\%$  for near-ambient concentrations. The atmospheric concentrations were determined relative to calibration standards prepared by dilution of certified commercially available standards<sup>1,2</sup>. For most of this study we used a single high-pressure standard. A study of the three calibration tanks used since 1984 showed that concentrations of  $H_2$  in these tanks did not change by more than  $-0.2\%$  or about  $-1$  parts per  $10^9$  by volume per year ( $-1$  p.p.b.v.  $yr^{-1}$ ), much smaller than the trends in the data.

Our data are monthly averaged concentrations at each site between October 1985 and April 1989 from  $\sim 3,500$  weekly air samples. To estimate the trends we first subtracted the seasonal cycle at each site<sup>3,4</sup>. The seasonal cycles, shown in Fig. 1*b*, are

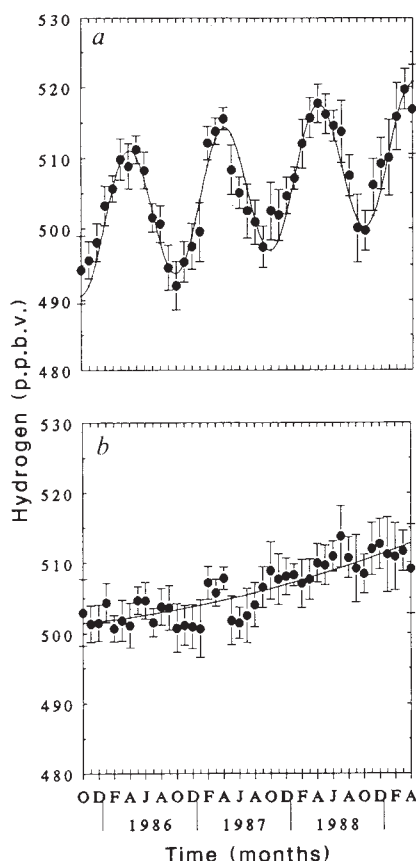


FIG. 2 Global average concentration of  $H_2$  including seasonal cycles (*a*) and without seasonal cycles (*b*). Vertical bars are 90% confidence limits. The curve in *a* is  $C(\text{p.p.b.v.}) = 500 + 8.2 \sin(2\pi t/12 - \pi/2)$  where time  $t$  is 0 at October 1985.

unusual in that the concentrations in the two hemispheres are not six months out of phase as is the case for most gases<sup>5</sup>. The seasonally adjusted concentrations of  $H_2$  at the six ground-level sites are plotted in Fig. 1*a*. As the concentration of  $H_2$  does not vary much with latitude, the data from different sites overlap.

We calculated a global average concentration for each month using

$$\bar{C} = \int_{-\pi/2}^{\pi/2} C(\phi) \cos(\phi) d\phi \approx \sum_{i=1}^6 a_i C_i \quad (1a)$$

$$\sigma_C^2 = \sum_{i=1}^6 a_i^2 \sigma_{C_i}^2 \quad (1b)$$

where  $\phi$  is the latitude, the  $C_i$  are the concentrations at the six sites starting with Alaska as  $i = 1$ ,  $\sigma_{C_i}$  is the standard deviation, and  $a_i$  is 0.0848, 0.1543, 0.2392, 0.2504, 0.1890 and 0.0828 for  $i = 1-6$ . Figure 2 shows the increasing trend in the global average concentrations.

Trace gases affected by human activities are normally more concentrated in the Northern Hemisphere, but the average ground-level distribution of hydrogen is the opposite<sup>5,6</sup>. The difference in the concentrations between the northern and southern polar regions is  $24 \pm 5$  p.p.b.v., between the middle latitudes of the two hemispheres it is  $15 \pm 4$  p.p.b.v., and between the tropical latitudes it is  $14 \pm 3$  p.p.b.v. These features appear in Fig. 1 but are more clearly seen in Fig. 3. We believe that these differences are caused by the hemispherical asymmetry of the removal process<sup>6</sup>. These features have not been seen before, probably because long-term systematic measurements have not previously been used to look for latitudinal gradients<sup>7-9</sup>.

We used four statistical methods to estimate the trends at each site and in the 'de-seasonalized' global average<sup>3</sup>: ordinary

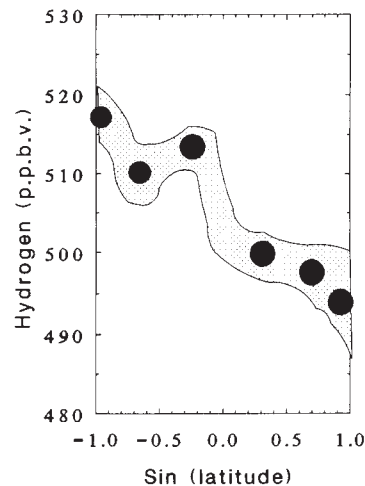
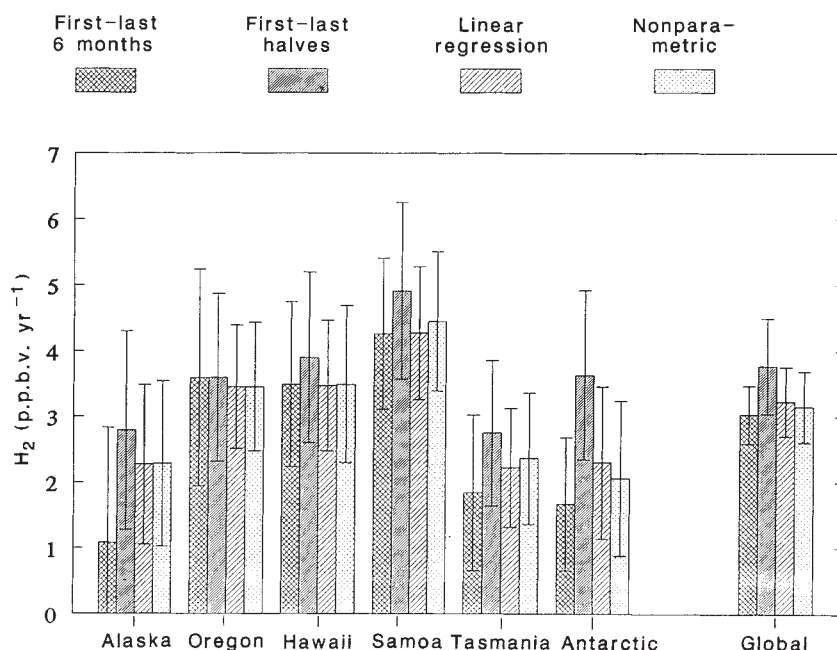


FIG. 3 The latitudinal variation of  $H_2$  at the Earth's surface. The shaded region is based on the 90% confidence limits of the mean concentrations at each site.

FIG. 4 Estimated trends of  $H_2$  using various statistical methods discussed in the text. Vertical bars are 90% confidence limits.



regression analysis, non-parametric trend analysis, comparison of the average concentration during the first half of the time series with the average concentration during the second half, and the first six-month average compared with the last six-month average<sup>3,10,11</sup>. In the last two methods the trend was calculated as  $[C_F - C_1]/\Delta T$ . When the first and second halves of the time series are compared,  $C_1$  is the average concentration in the first half of the time series,  $C_F$  is the average concentration in the second half and  $\Delta T$  is  $N/2$  where  $N$  is the number of months of data. For the comparison of concentrations during the first and last six months of the time series,  $C_1$  and  $C_F$  are the appropriate six-month average concentrations and  $\Delta T = N - 6$ . The uncertainties were estimated from the standard errors of  $C_F$  and  $C_1$ . The last two methods show directly that present concentrations are significantly greater than in the earlier period of sampling. Figure 4 shows that the different methods produce almost the same estimate for the trend, with small differences that are of no practical consequence.

The increasing trend of  $H_2$  is expected according to current understanding of the global hydrogen cycle<sup>12</sup>. The estimated sources and emission rates are:  $17 \text{ Tg yr}^{-1}$  directly from various anthropogenic activities,  $15 \text{ Tg yr}^{-1}$  from biomass burning,  $4 \text{ Tg yr}^{-1}$  from the oceans,  $29 \text{ Tg yr}^{-1}$  from methane oxidation,  $21 \text{ Tg yr}^{-1}$  from oxidation of non-methane hydrocarbons, and  $3-4 \text{ Tg yr}^{-1}$  from other smaller sources;  $78 \text{ Tg yr}^{-1}$  of  $H_2$  are removed from the atmosphere by soils and  $11 \text{ Tg yr}^{-1}$  by reacting with  $OH$ <sup>12-16</sup>. There are  $\sim 180 \text{ Tg}$  of  $H_2$  in the atmosphere, and it has an average atmospheric lifetime of  $\sim 2$  years. From this budget the increase cannot be attributed to changing removal processes. Recently most of the large sources of  $H_2$  have been increasing and therefore qualitatively explain the observed trend of atmospheric concentration. The increasing trend of methane alone may account for half of the observed trend of  $H_2$ . This budget indicates that  $H_2$  has been increasing for a long time, and pre-industrial levels may have been only around  $200 \text{ p.p.b.v.}$  Because the oxidation of hydrogen contributes substantial amounts of water vapour to the stratosphere, increasing hydrogen may already be contributing to increases in stratospheric water vapour. □

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ACKNOWLEDGEMENTS. We thank NOAA/GMCC for collecting samples at Alaska, Hawaii, Samoa, and the South Pole, P. J. Fraser for collecting samples at Cape Grim and D. Stearns for laboratory analyses. This work was funded by the NSF, Biospherics Research Corp. and Andaz Co.

## Role of solvent on vibrational energy transfer in solution

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ADVANCES in pulsed laser technology have made available ultra-short, tunable light pulses which permit the study of ultrafast energy-transfer processes within and between molecules. This information is essential for an understanding of chemical reactions on a molecular scale. Here we report results of a time-resolved study of the transfer of vibrational energy after excitation of the C-H stretch vibration in chloroform ( $CHCl_3$ ) and bromoform ( $CHBr_3$ ) dissolved in polar (deuterated acetone,  $CD_3COCD_3$ ) and non-polar (carbon tetrachloride,  $CCl_4$ ) solvents. We obtain quantitative information on the influence of the solvent on the rates of ultrafast intra- and intermolecular vibrational energy transfer. We find that the dipole-dipole interaction between an excited molecule and polar solvent molecules can lead to a strong acceleration of intramolecular energy-transfer processes. These results provide some insight into the way in which the rate of energy transfer and chemical reactivity are influenced by the polarity of the molecular environment.

In our experiment, a significant fraction of the molecules is directly vibrationally excited with an intense infrared pulse (pump). These excited molecules can no longer absorb infrared light of the same frequency and therefore the sample will be more transparent as long as the excited molecules have not relaxed to their initial state. The time-dependence of the

Received 27 July; accepted 22 August 1990.

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TABLE 1 Exponential relaxation time constants (ps).

| Solvent:<br>Process: | CCl <sub>4</sub> |          | CD <sub>3</sub> COCD <sub>3</sub> |        |
|----------------------|------------------|----------|-----------------------------------|--------|
|                      | IVR              | IET      | IVR                               | IET    |
| CHCl <sub>3</sub>    | 30 ± 2           | 140 ± 5  | 6 ± 1                             | 15 ± 2 |
| CHBr <sub>3</sub>    | 56 ± 2           | 260 ± 10 | 5 ± 1                             | 19 ± 2 |

IVR, internal vibrational relaxation; IET, intermolecular energy transfer.

relaxation can thus be determined by measuring the transmission of a weak pulse of the same frequency (probe) as a function of the delay with respect to the pump pulse<sup>1</sup>. When pump and probe overlap in time (delay zero), the transmission of the probe will be increased owing to the increase in transparency of the sample induced by the pump. For larger delays the transmission decreases owing to relaxation.

Our laser system generates tunable infrared pulses with a pulse duration of ~19 ps and an energy of ~200  $\mu\text{J}^{2,3}$ . The shot-to-shot stability of the spatial profile and the spectrum of the pulses are very important for the accurate measurements, which is essential in the study of dilute systems because of the small changes in the transmission of the probe pulse.

For the four combinations of solute and solvent we performed an extensive study in which we tuned the central frequency of the pulses through the whole C-H stretch absorption band. Figures 1 and 2 show typical results for CHCl<sub>3</sub> in CCl<sub>4</sub> (0.1M) and CHBr<sub>3</sub> in CD<sub>3</sub>COCD<sub>3</sub> (0.05M).

If the central frequency of the laser pulse is lower than the maximum of the absorption band (Figs 1b, 2b), we observe an overshoot of the decrease in transmission. This overshoot implies that for certain delays the absorption of the probe is even larger after excitation by the pump pulse than before. This indicates that

during the relaxation the transition frequency of the C-H stretch vibration of the excited molecules shifts to a lower value and into better resonance with the spectrum of the probe. Such a shift of 10–15  $\text{cm}^{-1}$  is very likely due to the fact that the relaxation leads to the excitation of other vibrations of the molecule that are anharmonically coupled with the C-H stretch vibration. After this relaxation the C-H stretch vibration can be excited again with a larger absorption coefficient if the central frequency of the pump and probe pulse was initially sufficiently below the maximum of the absorption band. For larger delay times we observe that the transmission rises again to its initial value if CCl<sub>4</sub> is used as solvent and to a constant increased transmission level if CD<sub>3</sub>COCD<sub>3</sub> is used as solvent. These observations indicate that in both solvents the relaxation takes place by two consecutive processes, which agrees with results of previous studies of CHBr dissolved in CCl<sub>4</sub><sup>4–6</sup>.

A possible explanation for the observation of an increased transmission level for large delay times with CD<sub>3</sub>COCD<sub>3</sub> as solvent is that the second relaxation process leads to a rise in temperature and that in this solvent the absorption band of the C-H stretch vibration depends on the temperature. We studied the temperature dependence of the absorption band of the C-H stretch vibration of CHCl<sub>3</sub> and CHBr<sub>3</sub> dissolved in CCl<sub>4</sub> and CD<sub>3</sub>COCD<sub>3</sub> by measuring infrared spectra at different temperatures with a conventional spectrometer. We observed very little effect with CCl<sub>4</sub> as solvent and a significant steepening of the low-frequency side of the absorption band with rising temperature with CD<sub>3</sub>COCD<sub>3</sub> as solvent<sup>7</sup>. This last observation can be understood from the fact that the strong dipole-dipole interactions between solute and polar solvent influence the transition frequency of the C-H stretch vibration and that the influence of these interactions becomes smaller when the temperature increases. In the second relaxation process the energy is

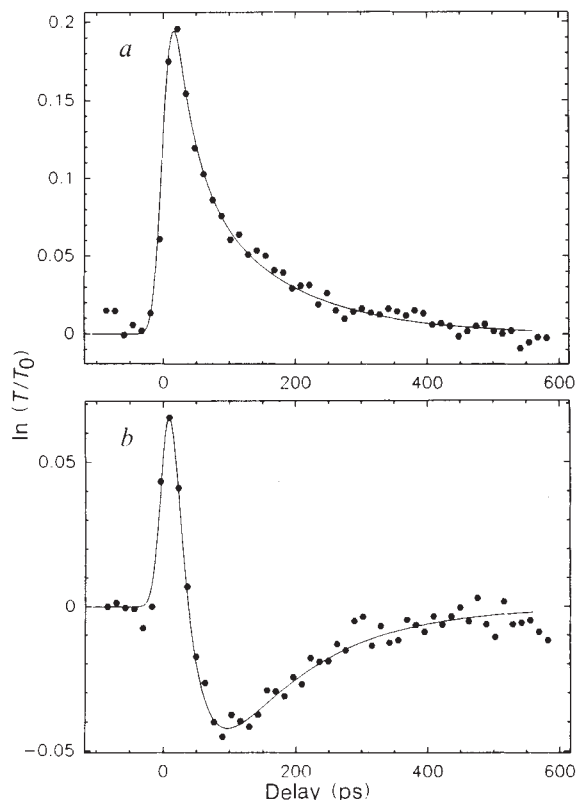


FIG. 1 Relative transmission of an infrared probe pulse as a function of the delay between probe and pump pulse for the C-H stretching mode of CHCl<sub>3</sub> dissolved in CCl<sub>4</sub> ( $\nu_{\text{max}} = 3,022 \text{ cm}^{-1}$ ) for two different central frequencies. Pump and probe have the same frequency spectrum. The numerically calculated result using the time constants of Table 1 is represented by the solid curve. a,  $3,024 \text{ cm}^{-1}$ ; b,  $3,014 \text{ cm}^{-1}$ .

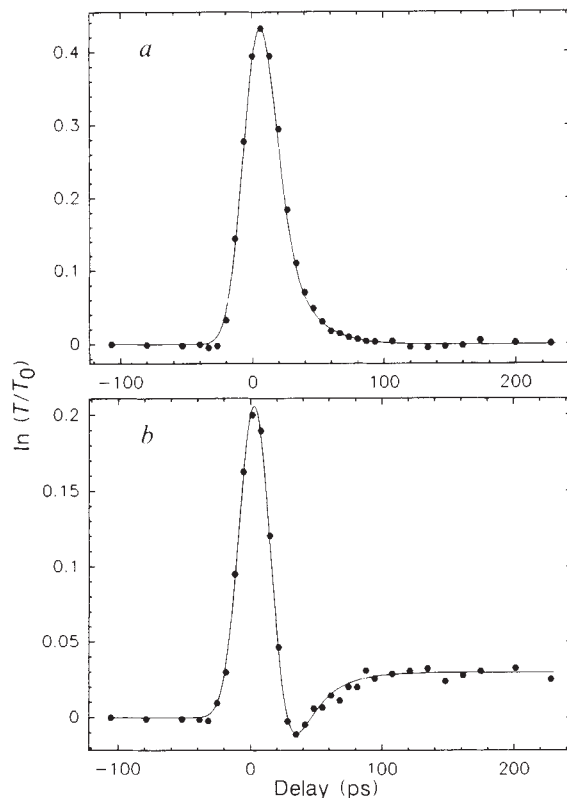


FIG. 2 Relative transmission of an infrared probe pulse as a function of the delay between probe and pump pulse for the C-H stretching mode of CHBr<sub>3</sub> dissolved in CD<sub>3</sub>COCD<sub>3</sub> ( $\nu_{\text{max}} = 3,028 \text{ cm}^{-1}$ ) for two different central frequencies. Pump and probe have the same frequency spectrum. The numerically calculated result using the time constants of Table 1 is represented by the solid curve. a,  $3,033 \text{ cm}^{-1}$ ; b,  $3,016 \text{ cm}^{-1}$ .

apparently transferred to the surrounding solvent molecules which results in an increase in the local temperature. The increased transmission level (Fig. 2b) remains constant on the picosecond timescale because the solvent molecules lose their energy by diffusion which typically takes place on a nanosecond or a microsecond timescale<sup>8</sup>.

We conclude that the relaxation takes place by two consecutive relaxation processes. In the first, intramolecular vibrational relaxation (IVR), the vibrational energy is transferred to other vibrations of the molecule. In a second, intermolecular energy transfer (IET) process, these other vibrations transfer the energy to the solvent molecules.

If the central frequency of the laser pulses is as high as or higher than the maximum of the absorption band (Figs 1a, 2a), the transition shifts out of resonance with the probe pulse after the internal vibrational relaxation process. The result is that the transmission of the probe remains increased as long as the molecules have not relaxed by intermolecular energy transfer. After this IET process the transmission of the probe returns to its initial value for both solvents. In this case we do not observe an increased level of transmission of the probe with  $\text{CD}_3\text{COCD}_3$  as solvent because a rise in temperature hardly changes the high-frequency side of the C-H stretch absorption band.

The time constants of both the internal vibrational relaxation and the intermolecular energy transfer can be determined by comparing the experimental results with numerical calculations. We calculate the amount of absorption of pump and probe in the sample using a simple kinetic model. In this model the ground and excited state of the C-H stretch with and without excitation of the other molecular vibrations are all modelled as single levels. These levels are coupled by the pump and probe pulses and the two relaxation processes. We calculate the total transmitted energy of the probe as a function of the delay between pump and probe. The calculations use as input the pulse parameters of pump and probe and as fit parameters the absorption coefficients and the exponential time constants of the relaxation processes. It turns out that it is even possible to determine accurately time constants that are much smaller than the pulse duration if the two relaxation processes have an opposite effect on the transmission of the probe (Fig. 2b), because in that case the functional form of the transmission of the probe pulse strongly differs from that of the wings of the pulses.

In a previous experiment<sup>9</sup>, in which the signal-to-noise ratio and the dynamic range were rather low, one much shorter time constant for the relaxation of the C-H stretch vibration of  $\text{CHCl}_3$  dissolved in  $\text{CCl}_4$  was determined using a different technique. The time constants for  $\text{CHBr}_3$  dissolved in  $\text{CCl}_4$  have been measured with essentially the same technique that we use<sup>4-6</sup> and with a different technique<sup>10</sup>. These values are in good agreement with our results.

The rate of IVR is proportional to the anharmonic coupling matrix elements between the excited vibration and near-resonant combinations of other molecular vibrations. In a liquid, this rate is strongly accelerated by the intermolecular interactions because these interactions allow excess energy of the excited vibration with respect to the combinations to be transferred to rotational and translational energy. These interactions can be viewed as fluctuations of the vibrational energy levels. During some fluctuations the coupling levels obtain the same energy and the energy transfer becomes possible<sup>11</sup>. In a non-polar solvent like  $\text{CCl}_4$  these fluctuations are small so that the fraction of fluctuations during which energy transfer becomes possible strongly depends on the energy differences between the levels. The observation that in  $\text{CCl}_4$  the time constant of the IVR is twice as large for  $\text{CHBr}_3$  than for  $\text{CHCl}_3$  can thus be understood from the fact that the energy difference between the C-H stretch vibration and the other vibrations is larger in  $\text{CHBr}_3$  than in  $\text{CHCl}_3$ . With  $\text{CD}_3\text{COCD}_3$  as solvent the fluctuations induced by the strong dipole-dipole interactions with the solvent

molecules can be large compared to the energy differences between the near-resonant levels. Therefore the rates of IVR of  $\text{CHCl}_3$  and  $\text{CHBr}_3$  are significantly accelerated and become approximately the same because the comparatively small energy differences no longer determine the fraction of fluctuations during which energy transfer becomes possible. □

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ACKNOWLEDGEMENTS. This work was supported by the Nederlandse Organisatie voor Wetenschappelijk Onderzoek (Netherlands Organization for the Advancement of Research).

## Nanometre-scale chemical modification using a scanning tunnelling microscope

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**THE potential of the scanning tunnelling microscope (STM) as a tool for the high-resolution manipulation of surfaces and surface-adsorbed phases has been amply demonstrated<sup>1-5</sup>. In general, previous studies have been concerned with the physical rearrangement of surface atoms and molecules. Here I report the use of the STM to achieve chemical modification of a surface. An STM is used to etch the surface of a mixed-ionic conductor ( $\text{Ag}_x\text{Se}$ ), producing selected patterns of grooves about 10 nm wide. The etching process seems to involve the segregation of different chemical species on the surface: silver atoms migrate into the semiconductor matrix that underlies the  $\text{Ag}_x\text{Se}$  film, exposing selenium ions which are then removed from the surface by reaction with ambient hydrogen. Chemical modification of this sort may come to play a part in future nanometre-scale technologies.**

Materials that are susceptible to compositional changes have chemical and thermodynamic properties that vary considerably with the external conditions. This is in contrast to, for instance, semiconductors and metals, for which the chemical equilibria are fixed. In solid electrolytes, however, changes in chemical potential of the ionic species usually cause ions to move about in the matrix, resulting in chemical modification. The other essential requirement for localized chemical modification using an STM is a material with a high electronic conductivity.

Here I have used a silver selenide compound because it is a typical mixed-ionic and electronic conductor. Its stoichiometric composition,  $\text{Ag}_2\text{Se}$ , has a high ionic conductivity as well as a high electronic conductivity, even at room temperature<sup>6</sup>. These conductivities are generally preserved even in slightly non-stoichiometric compositions. The Ag-Se compound was prepared by silver-plating a  $\text{Ge}_{0.1}\text{Se}_{0.9}$  film that had been deposited on the substrate using radio-frequency sputtering. (The small amount of germanium was added to impede microcrystallization of the Se network.) The films, 250-500 nm thick, were then immersed in a solution containing  $\text{Ag}(\text{CN})^{-2}$  anions (pH 12) for a few minutes. Here I am concerned with the molecular reaction that produces silver selenide on a scale of 1 nm or less<sup>7</sup>. By repeatedly alternating Auger analysis and surface stripping



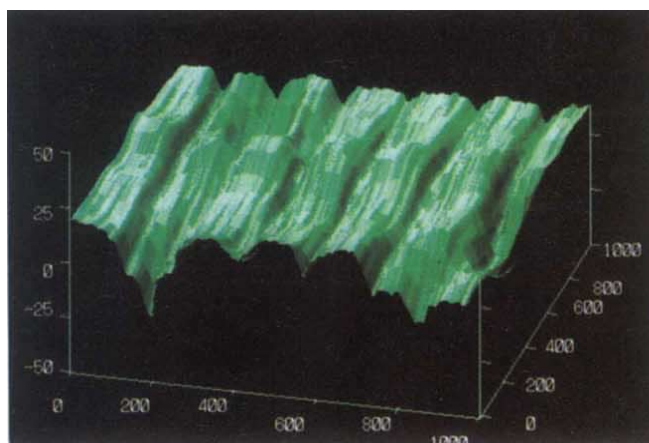


FIG. 1 STM topograph of the corrugated grating formed on  $\text{Ag}_x\text{Se}$  surface. Distances are in nanometres. Tip was scanned at a speed of  $0.2 \text{ nm ms}^{-1}$  ( $x$  direction) with a pitch of  $2.5 \text{ nm}$  ( $y$  direction). Tip bias was  $+3.5 \text{ V}$ .

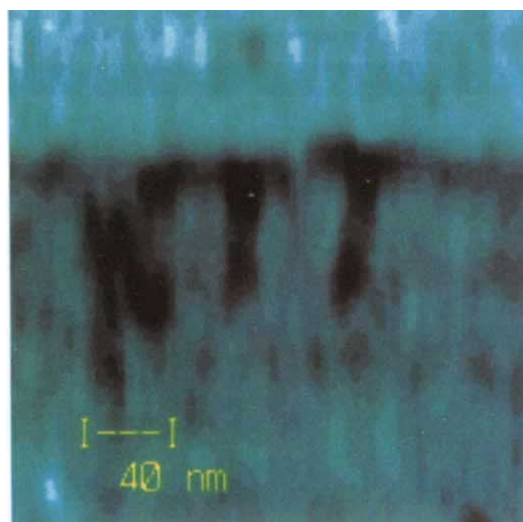


FIG. 2 STM image of nanometre-sized letters NTT written on  $\text{Ag}_x\text{Se}$  surface. The frame is  $235 \times 235 \text{ nm}$ . Each letter is  $\sim 40 \text{ nm}$  wide and  $70 \text{ nm}$  long. The lines are  $13 \text{ nm}$  wide and etched to a depth of  $\sim 3 \text{ nm}$ . Tip bias was  $+3.0 \text{ V}$ .

(by immersion in aqua regia) I found that the surface of the film was  $\text{Ag}_x\text{Se}$  ( $x = 1.9$ ), up to a thickness of  $\sim 15 \text{ nm}$ .

Platinum tips with spherical ends (as revealed by scanning electron microscopy) produced the atomic-scale STM images. To apply voltage to the  $\text{Ag}_x\text{Se}$  surface, a silver electrode was attached to part of it. The sample system thus constitutes a cell, having an ion-blocking electrode composed of the Pt tip and a gap between the tip and the sample surface. The microscope was generally operated in constant-current mode for imaging and modification. All surface images were obtained at a current of  $0.1 \text{ nA}$  with bias voltage of  $+1.0 \text{ V}$  (the polarity of bias refers to the tip).

First, I performed a modification experiment to fabricate a coarse and deeply grooved grating. After confirming the flatness of the  $\text{Ag}_x\text{Se}$  surface, the tip was scanned over the prescribed areas of a computer-generated template (a grating pattern with lines  $60 \text{ nm}$  wide and spaces  $100 \text{ nm}$  wide) using a constant

current of  $0.1 \text{ nA}$ , in air. Immediately after the modification procedure, the STM image of the surface was obtained. When the bias voltage for the modification was raised to a few volts, the images of the surface were altered. Figure 1 shows clear surface corrugation obtained using a bias of  $+3.5 \text{ V}$ . A cross-section of the image revealed that areas traversed by the tip are deeply etched, with grooves (average depth  $15 \text{ nm}$ ) comparable to the thickness of the  $\text{Ag}_x\text{Se}$  layer itself.

Figure 2 shows that nanometre-sized letters can be generated and that the tip can be dexterously manipulated throughout the writing procedure. To write the letters 'NTT', I again used computer-generated templates. According to a profile analysis, the lines are grooves. Using a d.c. voltage, I could produce continuous lines, but with pulse-voltage techniques<sup>3,4</sup> I could not. Significantly, I had little success when I tried to induce structures on the surface using a lower bias voltage of  $+1 \text{ V}$ , even for currents as high as  $2 \text{ nA}$ .

Preliminary experiments revealed that the current is exponentially related to the gap width between the tip and the  $\text{Ag}_x\text{Se}$  surface. This proves that the controlled current in this system is almost completely dominated by electronic, not ionic current, which means there is no contact between the tip and the surface during the imaging and modification operations. This is different from the usual solid electrolytes (ionic conductors)<sup>5</sup>.

Apparently the surface was not modified by abrasive etching<sup>5</sup>. Local heating is also highly improbable, considering the above high-current experiment. By default, an electric-field effect is most probable. The dependence on field strength and bias polarity was studied by performing experiments in air without scanning the tip over the surface, thus forming a concavity on the surface (Fig. 3a). After these experiments, the tip was able to produce atomic-resolution images of a graphite surface, showing that no material had been lost from the tip (which, in constant-current mode, might have provided another explanation for the apparent concavity). Figure 3b shows the time-dependence of the depth of the concavity for biases of  $1$ – $5 \text{ V}$ . A bias of  $1 \text{ V}$  produces no effects, a bias of  $2 \text{ V}$  yields a small effect, and a bias over  $3 \text{ V}$  readily digs up the surface. Surprisingly, this digging persists even beyond the thickness of the  $\text{Ag}_x\text{Se}$  layer ( $\sim 15 \text{ nm}$  thick), that is, into the underlying Ge-Se layer. Thus, the applied field is responsible for the digging. Another important consideration is the polarity effect. When a negative bias ( $-1$  to  $-5 \text{ V}$ ) was applied to the tip, no concavity was produced on the surface at all. Owing to the high ionic conductivity of

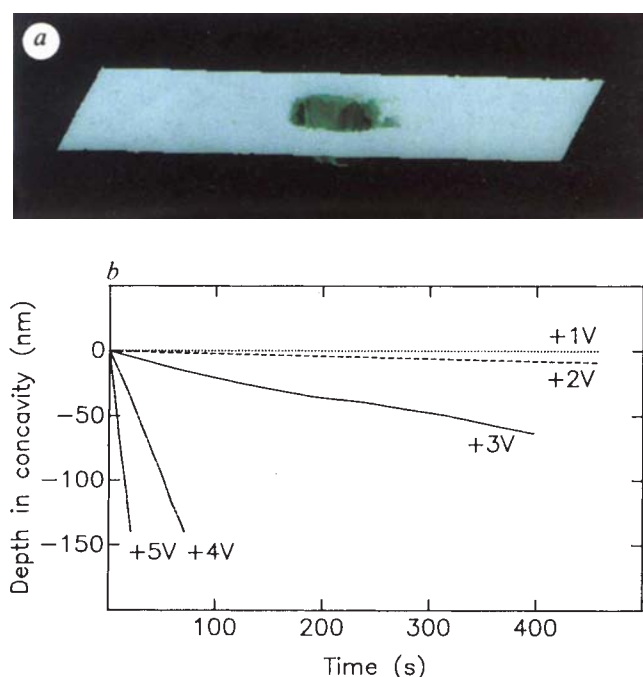


FIG. 3 Concavity characteristics formed without  $x$ - $y$  scanning of the tip in air. *a*, STM image of a concavity. The frame is  $500 \times 500 \text{ nm}$ . *b*, Time-dependence of the depth of the concavity. Characteristics were obtained by recording the change in voltage of the feedback loop in constant-current mode, using currents of  $0.1 \text{ nA}$ . Tip bias was varied from  $+1$  to  $+5 \text{ V}$ .

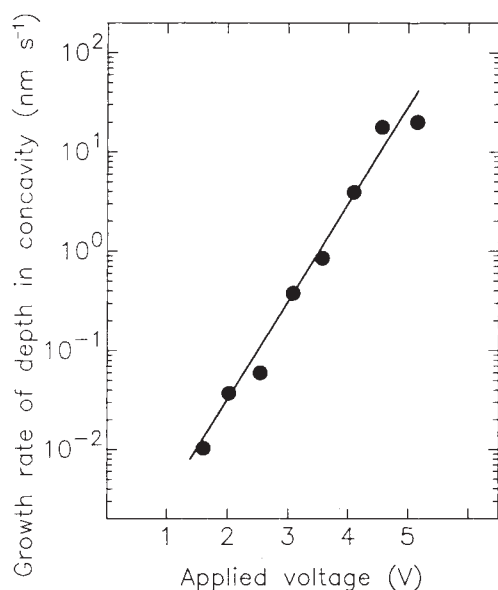
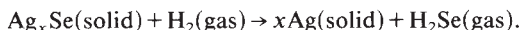


FIG. 4 Dependence on applied voltage of the growth rate of the depth of the concavity formed in hydrogen gas (1 atm). Data were deduced from the time-dependence of the depth in parameter of tip bias (+1 to +5 V), similar to the results shown in Fig. 3b.

silver selenide, it seems probable that a positive bias to the tip provides a flux of mobile  $\text{Ag}^+$  ions from the surface toward the underlying matrix (Ag migration), resulting in segregation of the Ag and Se. Increasing the bias seems to accelerate the segregation. This is impossible with a negative bias. Therefore, I believe that etching involves segregation of the  $\text{Ag}_x\text{Se}$  by the field, exposing the surface composed of immobile negative ions ( $\text{Se}^-$ ) to atmosphere.

Wagner *et al.* pointed out that when silver sulphide is electrolysed in hydrogen gas, it is reduced:  $(\text{Ag}_2\text{S} + \text{H}_2 \rightarrow 2\text{Ag} + \text{H}_2\text{S})^{9,10}$ . The formation rate of  $\text{H}_2\text{S}$  molecules,  $n_{\text{H}_2\text{S}}$ , depends exponentially on the applied field  $E$ ,  $E \propto \ln n_{\text{H}_2\text{S}}$ . Environmental effects were measured at various biases, yielding the results (for air) shown in Fig. 3. Although no concavity formation was observed even for bias application up to +5 V in vacuum ( $2 \times 10^{-3}$  torr), in the presence of nitrogen and oxygen gases (1 atm), hydrogen gas (1 atm) caused concavities and high-speed changes in feedback voltage exceeding that in air, as expected. Figure 4 shows the dependence on applied voltage of the depth of concavity formed in hydrogen gas. Hydrogen atoms are readily available as  $\text{H}_2\text{O}$  molecules in air, so one can reasonably predict a substantial STM-induced reduction reaction on the segregated  $\text{Ag}_x\text{Se}$  surface



I assume that the volume of the tip at the top,  $V$ , is associated with the number of  $\text{H}_2\text{Se}$  molecules formed,  $n_{\text{H}_2\text{Se}}$ . The surfaces were concave beyond a depth of 150 nm. This means the tip depth is roughly equivalent to the length from the spherical tip,  $b$ . If  $R$  is the radius of the spherical tip, the volume is  $V = 2\pi Rb^2$ , because usually  $R \gg b/3$ . Consequently,  $E \propto \ln b$ .

Figure 4 shows that the increasing depth of the concavity is an exponential function of the applied voltage. This agrees well with the above relation, being associated with the formation of  $\text{H}_2\text{Se}$  molecules. Thus the etching in the  $\text{Ag}_x\text{Se}$  surface is probably attributable to a reduction reaction of the Se surface, the Se made available by the segregation of  $\text{Ag}_x\text{Se}$  by the field. I am now trying to pinpoint the whereabouts of residual Ge atoms.

Vertical chemical modification is promising as a nanolithography technique for fabricating nanometre-scale devices, say in combination with techniques of lateral atom/molecule manipulation<sup>1,2</sup>. The  $\text{Ag}_x\text{Se}/\text{Ge}-\text{Se}$  system has already shown a potential for recording X-ray holograms and fabricating large-

scale integrated circuits<sup>11,12</sup> as a submicrometre-scale lithography resist; such applications might now be feasible on nanometre scale. □

Received 18 June; accepted 6 September 1990.

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ACKNOWLEDGEMENTS. I thank T. Nagamura for his help.

## Effect of magnetic fields on a propagating reaction front

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SOME autocatalytic reactions may exhibit propagating reaction fronts if a small amount of autocatalyst is introduced into the unstirred, autocatalyst-free system<sup>1–10</sup>. The velocity of the front propagation depends on the diffusion constants of the reacting species, on their initial concentrations, on the kinetics and mechanism of the reaction and on any electric<sup>11,12</sup> and gravitational forces<sup>13</sup> that influence the migration of the reactive species. The cobalt(II)-catalysed auto-oxidation of benzaldehyde in glacial acetic acid<sup>14</sup>, which involves radicals as well as the transformation of paramagnetic Co(II) into diamagnetic Co(III), is an example of a propagating-front reaction<sup>15</sup>. It is well known<sup>16,17</sup> that magnetic fields may alter the rate constants of some radical reactions by ~5–30%. Here we present results which show that, in this system, even a weak magnetic interaction may cause a change of several orders of magnitude in the velocity of the propagating front, as a result of the paramagnetic–diamagnetic transformation involved in the process.

Stock solutions and other chemicals were prepared and purified as previously reported<sup>15</sup>. The experiments were carried out at room temperature ( $22 \pm 2^\circ\text{C}$ ) in a glacial acetic acid solution of 0.0083M (saturated)  $\text{O}_2$ , 0.98M benzaldehyde and 0.0075M Co(II) acetate. The front velocity was followed by eye along the horizontal in capillary tubes of 2-mm inner diameter and also in 2-mm-deep layers enclosed between air-tight glass plates of various sizes. The reactions were initiated by adding a drop of 0.05M perbenzoic acid, dissolved in glacial acetic acid, at the end of the capillary tube or at a 1-mm hole drilled in the centre of the upper plate. The perbenzoic acid caused the pink colour of the Co(II) to change to green Co(III) and this front started to travel along the tube or in the quasi two-dimensional reaction vessel. The magnetic field strength for different positions of the magnet poles was measured at grid points 2.5 mm apart using a Hall probe.

We carried out the first set of experiments in capillary tubes between flat cylindrical poles (diameter 10 cm) 8 cm apart. The capillary tube was placed directly beside the face of one of the poles and the front initiated outside the field. The front started to propagate with a velocity of  $3 \text{ mm min}^{-1}$ . Before reaching the edge of the pole, however, the front slowed down, stopped and

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stayed there for up to 8 h. When the electromagnet was switched off, the front immediately started to travel. If the reaction front was initiated between the poles, it started to travel with the usual velocity until it approached the edge from inside. Before reaching the edge it slowed down and, again, stopped. When the magnet was switched off, the front started to travel with the original velocity. We then placed the capillary tube at equal distances from the poles and initiated the reaction outside the field. As before, the front slowed down and stopped. When we initiated the front inside the poles, however, it accelerated by a factor of about five as it reached the edge. At about 5–10 cm from the edge, it slowed down to the normal velocity.

We then carried out experiments in a quasi two-dimensional arrangement, a thin (2 mm) flat, rectangular (80 × 180 mm) reaction vessel. With the electromagnet off, the front was initiated at the hole. We allowed the circular front to travel for two minutes and then switched on the magnet. No further displacement of the front towards the poles occurred. The velocity in the  $y$  direction, however, increased and an elongated structure developed in ~15 min. The temporal development of the front is illustrated in the bottom of Fig. 1. The magnetic field strength measured in the  $x$ - $y$  plane is plotted in the upper part of the Fig. 1, together with the development of the boundary of the front projected to the field-strength surface.

Comparing the field-strength surface with our results, the front seems to be unable to propagate against increasing magnetic field. It chooses the direction of the 'steepest descent' in the magnetic field and is accelerated when it gets into a 'downhill' region.

We then studied the system in the more complex, asymmetric magnetic field generated by a combination of flat and conical poles. Figure 2 shows the temporal development of the front when it was initiated in the middle of a quasi two-dimensional vessel (width 3.0 cm, length 18.0 cm). The front quickly propagates towards the flat pole, then travels along the edge of the

vessel but avoids the two small magnetic 'hills'. Finally, the reacted zone slowly increases in all directions at the 'bottom'. The results suggest that the inhomogeneity of the field, not the field strength itself, govern the observed phenomena. To prove this, the flat poles were placed only 1 cm apart in the following experiment. In this configuration, the magnetic field is homogeneous to within 0.2% in the inner 8 cm length and the field strength does not increase at the edges. The front initiated inside this field travelled with a constant velocity, which was 25% higher than the velocity measured in a field-free experiment using the same solution. The front accelerated by a factor of about five when it passed the edge, then slowed down to the velocity measured in the control experiment. Thus a homogeneous field may change the rate constants, but does not stop the reaction front.

To explain the results, we must take into account that the unreacted solution before the front is paramagnetic because of the presence of Co(II) and dissolved oxygen. This part of the system is always attracted towards the stronger field. The reacted solution is diamagnetic and is repelled, towards the weaker magnetic field. The situation is somewhat similar to the gravity-induced anisotropies of propagating fronts<sup>13,18</sup>. The difference is that gravity exerts an attractive force on both the reacted and unreacted zones, although these forces—normalized to unit volume—are different because of the difference in densities. Therefore, the gravity cannot stop the front; there is a limit, the gravity-independent 'pure' reaction diffusion velocity, below which the velocity of the front can never decrease. Recent considerations<sup>19</sup> have shown that the front velocity in a gravitational field is the sum of the reaction diffusion velocity and the convection velocity. In the inhomogeneous magnetic field, however, there are attractive and repulsive forces. In our system, when the front travels in the direction of decreasing field, the reacted zone is pushed away and the unreacted zone is attracted. Thus, significant convective motion may develop, explaining the acceleration of the front 'down' the magnetic field. When the front tries to travel in the direction of increasing field, the

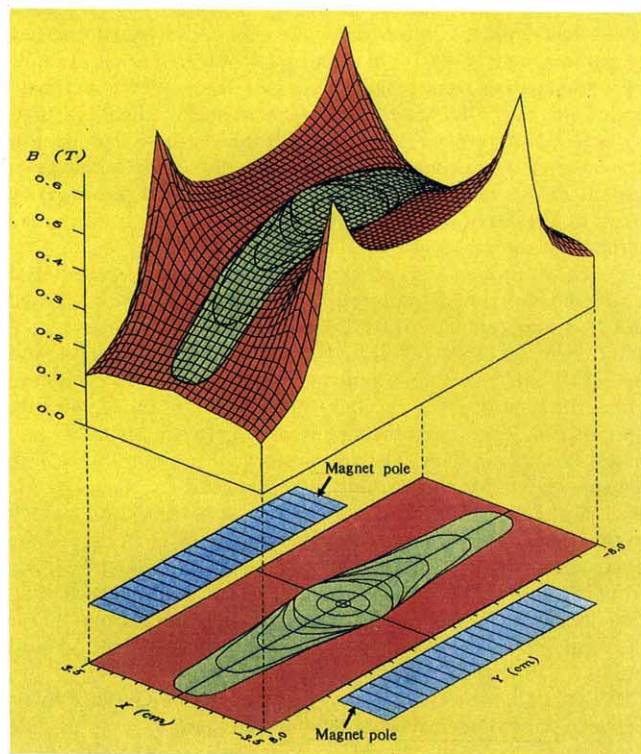


FIG. 1 The magnetic field strength in the central horizontal plane of two flat cylindrical poles (diameter 10 cm) when they are 8 cm apart ( $V=110$  V;  $I=12$  A), and the development of the front in the quasi two-dimensional system.

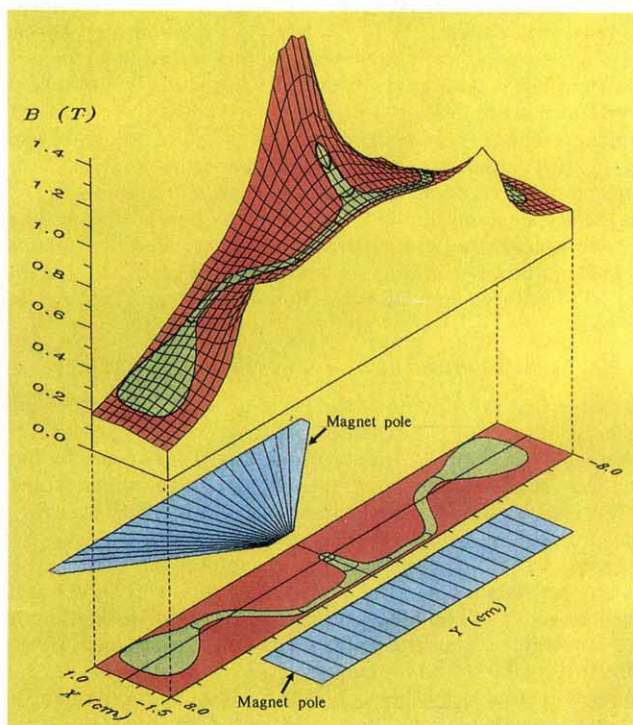


FIG. 2 The magnetic field strength in the central horizontal plane of one flat cylindrical and one conical pole when they are 3 cm apart, and the development of the front in the quasi two-dimensional system.

magnetic force acts against the diffusion. When the inhomogeneity is large enough, it may even stop the diffusion or at least decrease its rate to such an extent that no front propagation is possible. When we decreased the field by decreasing the current through the magnet, the front slowed down, but did not stop, when travelling 'up' the field. Also, the acceleration of the front was less in the 'downhill' regions.

The observations from our preliminary experiments are consistent with the above explanation. If we prepare a sample with a sharp boundary between non-reactive Co(II) and Co(III) solutions in a capillary tube and place it in the inhomogeneous part of the field with the Co(III) in the direction of lower field strength, no significant diffusion is observed for hours. The diffusion becomes noticeably faster when the magnet is switched off. The most surprising phenomenon is that if we switch on the magnet when the mutual diffusion is already evident, a sharp boundary between the two solutions re-forms. On the other hand, if we prepare a homogeneous solution of Co(II) and Co(III), we observe no separation of the species, even at the highest field gradient available with our electromagnet. Inhomogeneity of both solution and magnetic field are therefore essential.

Although the energy of magnetic interactions is several orders of magnitude smaller than the other energies involved in chemical processes, we have observed that such a weak interaction may cause a change of several orders of magnitude in a well defined, easily measurable physico-chemical parameter. We tentatively suggest that the reason for this enormous effect is that the forces between the magnetic field and the reaction front are directional, whereas the other forces are spatially averaged.

Our explanation could be tested by a propagating front in which one paramagnetic species is transformed to another as the front travels along. In this case, because the magnetic field would attract both reacted and unreacted zones, a pattern very similar to the one observed in the gravity effect<sup>13</sup> would be expected; the propagating front should not be stopped by the magnetic field.

This system is rather difficult to handle experimentally and the reproducibility rather poor<sup>15</sup>. We are therefore seeking new systems, in aqueous solution if possible, that are better candidates for quantitative study of this phenomenon. A promising candidate is the oxidation of Co(II)EDTA complex in aqueous solution by H<sub>2</sub>O<sub>2</sub>, because OH<sup>-</sup> ion is produced during the reaction and the reaction is accelerated by increasing pH. □

Received 27 June; accepted 10 September 1990.

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ACKNOWLEDGEMENTS. We thank Professors P. DeKemper and I. R. Epstein for comments on the manuscript. This work was supported by the Hungarian Academy of Sciences.

## Changes in carbon isotope ratios in the late Permian recorded in therapsid tooth apatite

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**THE end of the Permian witnessed global extinctions of marine and terrestrial fauna<sup>1</sup>. As marine biogenic carbonate and atmospheric carbon dioxide are in long-term isotopic equilibrium, changes in carbon isotope ratios in marine carbonate strata should record changes that occurred at that time in atmospheric ratios; one might then expect a correlation between <sup>13</sup>C/<sup>12</sup>C ratios in such marine sequences and those in terrestrial records that derive from carbon-bearing materials that incorporate atmospheric CO<sub>2</sub>. An example of the latter is fossil tooth apatite from herbivorous fauna. Correlations of this sort between marine and terrestrial records are, however, difficult to establish as they rely on particular characteristics of the isotope curves. Here we show that stable-carbon isotope ratios in tooth apatite from *Diictodon* (a mammal-like**

reptile), in the late Permian Lower Beaufort succession of the South African Karoo (~260-245 Myr), show a progressive depletion in <sup>13</sup>C that is very similar to trends in marine carbonates from late Permian deposits in Eurasia<sup>2-7</sup>. These results suggest that global changes in total biomass occurred towards the end of the Permian: primary production decreased and continental aridity increased<sup>8</sup>, leading to a depletion of <sup>13</sup>C in atmospheric and oceanic CO<sub>2</sub>.

Studies of stable carbon isotopes in fossil biological apatites have indicated the potential of using tooth enamel rather than bone for palaeoenvironmental and palaeodietary studies<sup>9-11</sup>. This method has been applied to analyses of Plio-Pleistocene ungulates from southern Africa<sup>10-12</sup> and Mio-Pliocene herbivores from Pakistan (J. Quade *et al.*, manuscript in preparation), demonstrating that carbon isotope ratios from fossil apatite retain a biogenic signal associated with consumption of C<sub>3</sub> or C<sub>4</sub> vegetation in palaeoenvironments. Here we apply this method to samples of *Diictodon* tooth apatite from five vertebrate assemblage zones in the terrestrial late Permian succession of the South African Karoo (Fig. 1), to determine whether stable-carbon isotope ratios show trends comparable to those in late Permian sequences of marine carbonates in Eurasia<sup>2-7</sup>.

The genus *Diictodon* has been chosen for analysis because it was herbivorous<sup>13</sup> and is represented in all of the late Permian vertebrate assemblage zones of the South African Karoo. As grasses with the C<sub>4</sub> pathway did not exist in the Permian, it is probable that the diet of *Diictodon* included plants with a C<sub>3</sub> photosynthetic pathway.

Fragments of fossil *Diictodon* teeth were cleaned mechanically to remove matrix and calcite crystals in the central core (former pulp cavity). Samples were ground and pretreated in 1M acetic acid<sup>11,12</sup>. Infrared spectroscopy confirmed that the material was apatite and that it contained no other carbonate phases (such as calcite) after acetic acid treatment. CO<sub>2</sub> was produced by digestion in 100% phosphoric acid and measured in a VG 602E



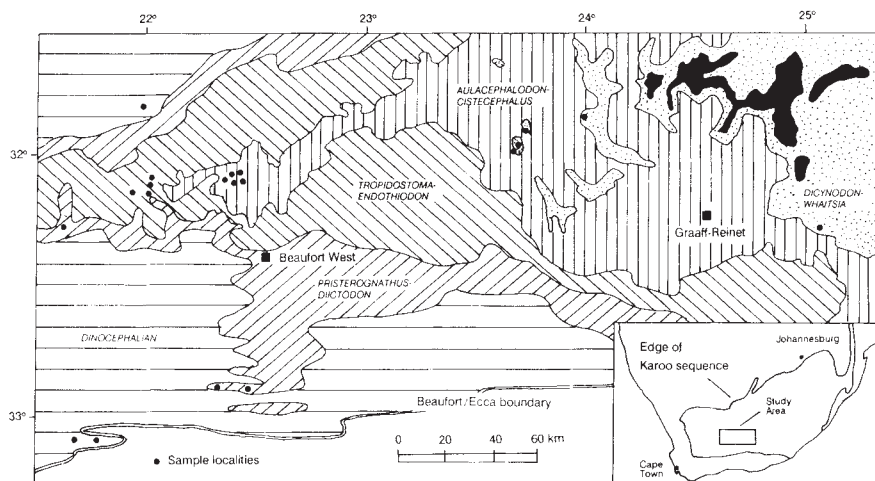


FIG. 1 *Diictodon* sample localities in the late Permian Lower Beaufort succession of the South African Karoo (260–245 Myr). Vertebrate assemblage zones indicated in italics; legend given in Fig. 2.

micromass spectrometer. The precision of the isotope ratios is  $<0.05\%$ .

Figure 2 shows a decrease in  $\delta^{13}\text{C}$  values (depletion in  $^{13}\text{C}$ ) towards the end of the Permian, similar in scale and direction to changes found in marine carbonates from Eurasia<sup>2–7</sup>. Mean  $\delta^{13}\text{C}$  values for late Permian samples from the *Pristerognathus/Diictodon* and *Tropidostoma/Endothiodon* assemblage zones (both  $-8.2\%$ ) are relatively more positive than those obtained for samples from younger zones in the sequence. Specimens from the *Aulacephalodon/Cistecephalus* assemblage zone has values clustered tightly around a mean of  $-10.6 \pm 0.3\%$ , contrasting with a mean  $\delta^{13}\text{C}$  of  $-14.4 \pm 1.3\%$  for samples from the *Dicynodon/Whaitsia* assemblage zone in which the abundance of *Diictodon* decreases relative to other vertebrate taxa.

The most positive  $\delta^{13}\text{C}$  values obtained from Dzhulfian–Dorashamian sediments in Eurasia, before the decline in  $\delta^{13}\text{C}$  beginning at  $\sim 250$  Myr (refs 3, 6), can be correlated with relatively positive  $\delta^{13}\text{C}$  values for *Diictodon* samples from the *Tropidostoma/Endothiodon* assemblage zone. An age estimate of  $\sim 250$  Myr for the latter is consistent with ages of  $\sim 260$  Myr for the lower boundary of the Beaufort succession<sup>14</sup> and  $\sim 245$  Myr for the Permo/Triassic boundary (Fig. 2).

Several factors are known to affect the  $\delta^{13}\text{C}$  value of terrestrial and marine records. Aridity is known to contribute to variability in  $\delta^{13}\text{C}$  in modern  $\text{C}_3$  plants<sup>15,16</sup>. A general decline of  $>6\%$  in this study of mammal-like reptiles is associated with evidence for increased aridity at a time when the Karoo was part of the Gondwana continent<sup>17,18</sup>. Berner<sup>8</sup> suggested that increased continental aridity was a primary factor in causing global changes in  $\delta^{13}\text{C}$  in late Permian terrestrial palaeoenvironments.

Irrespective of the extent to which temperature or other factors were responsible for aridification in the Karoo during the late Permian, the decrease in  $\delta^{13}\text{C}$  is likely to have coincided with decreased marine primary production.

Isotope ratios have not been obtained for calcite and matrix associated with all *Diictodon* samples, but several lines of evidence indicate that  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values for apatite from the fossil teeth are not simply a reflection of isotope exchange with deposition environments.  $\delta^{18}\text{O}$  values for *Diictodon* apatite, showing little variation overall (mean  $\delta^{18}\text{O} = -16.3 \pm 0.9\%$ ,  $n = 21$ ), do not correlate with  $\delta^{13}\text{C}$  values for the same samples (correlation coefficient,  $r^2 = 0.02$ ). In addition,  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values for calcite or matrix associated with *Diictodon* teeth do not correspond to those obtained for apatite from the same specimens. Notably, a  $\delta^{18}\text{O}$  value of  $-6.5\%$  for calcite from the core of a specimen from the *Tropidostoma/Endothiodon* assemblage zone differs significantly (probability  $p < 0.05$ ) from the mean  $\delta^{18}\text{O}$  value of  $-16.8 \pm 0.7\%$  ( $n = 5$ ) for apatite from specimens in the same zone, and a  $\delta^{13}\text{C}$  value of  $-13.5\%$  for the calcite differs significantly ( $p < 0.05$ ) from the mean  $\delta^{13}\text{C}$  value of  $-8.2 \pm 1.1\%$  ( $n = 5$ ) for apatite samples from this zone. Similarly, a  $\delta^{13}\text{C}$  value of  $-9.8\%$  for matrix associated with a sample from the *Dicynodon/Whaitsia* assemblage zone differs significantly ( $p < 0.05$ ) from the mean  $\delta^{13}\text{C}$  value of  $-14.4 \pm 1.3\%$  ( $n = 5$ ) for apatite from specimens in this zone.

$\delta^{13}\text{C}$  values more negative than  $-13.5\%$  (as found for apatite samples in the *Dicynodon/Whaitsia* assemblage zone) are rare in modern soil or palaeosol carbonates<sup>19–22</sup>, yet the mean  $\delta^{13}\text{C}$  value of  $-14.4 \pm 1.3\%$  for tooth apatite of end Permian  $\text{C}_3$  herbivores is comparable to the mean  $\delta^{13}\text{C}$  value of  $-14.5 \pm 1.6\%$  for modern  $\text{C}_3$  herbivore tooth apatite<sup>11</sup>, and the mean  $\delta^{13}\text{C}$

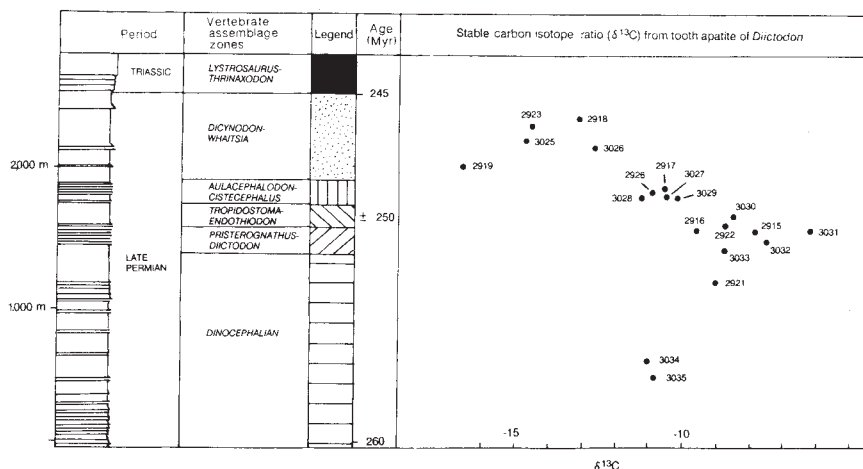


FIG. 2 Temporal variation in  $\delta^{13}\text{C}$  values measured from *Diictodon* tooth apatite in samples from five vertebrate assemblage zones in the late Permian Lower Beaufort succession of the South African Karoo. Sample numbers assigned by the Archaeometry Laboratory, University of Cape Town; specimens collected by staff of the S. Afr. Museum (Cape Town) and Geological Survey (Pretoria).  $\delta^{13}\text{C}(\text{‰}) = \{[(^{13}\text{C}/^{12}\text{C})_{\text{sample}} / (^{13}\text{C}/^{12}\text{C})_{\text{standard}}] - 1\} \times 1,000$  standard is PDB.

value of end Permian marine carbonates<sup>2-7</sup> is close to those of modern deep-ocean carbonates<sup>23</sup>. Although diagenesis may have contributed to variability in the absolute value of  $\delta^{13}\text{C}$  in fossil apatites, the similar scale and direction of change in marine and terrestrial sequences, from Northern and Southern Hemispheres, respectively, suggest that the  $\delta^{13}\text{C}$  values in fossil apatites reflect biogenic variation associated with global changes in  $\text{CO}_2$  and a reduction in primary production towards the end of the Permian. □

Received 21 May; accepted 30 August 1990.

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ACKNOWLEDGEMENTS. We thank T. E. Cerling, E. Cobabe, G. King and M. Magaritz for comments, and A. Crean for the preparation of *Diictodon* fossils. This study was supported by the Foundation for Research Development, South Africa, through a grant to N.J.vdM.

## Parallel long-term trends across four marine trophic levels and weather

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THE temporal changes revealed by long-term studies of components of the marine ecosystem<sup>1-4</sup> are of particular relevance given the growing concern about trace-gas-induced climatic change<sup>5</sup>. The extent to which they reflect the signal of weather relates directly to the validity of using them for detecting climatic change<sup>6-8</sup>. We report here on remarkable similarities in trends among four series of marine data and one of climatic data. The series overlap in time over 33 years (1955-1987), and in space over the northwestern quadrant of the North Sea. They relate to the abundances of phytoplankton, zooplankton and herring (*Clupea harengus*), to the breeding performance of the kittiwake gull (*Rissa tridactyla*), and to variations in weather pattern. The mechanisms behind the parallelism in trends remain unclear; a major challenge is to construct food-web models capable of reproducing the pattern and thereby of use in interpreting and detecting global climatic change.

The plankton data were collected under the Continuous Plankton Recorder survey<sup>9</sup>, a routine monthly synoptic survey of phytoplankton and zooplankton of the North Atlantic and North Sea carried out since 1948. The patterns of annual variability common to all taxa of phytoplankton and of zooplankton were represented by the first principal component of the standar-

dised mean taxon abundances per calendar year, for the area bounded by the north-east coast of Britain, between latitudes 55°N and 58°N, to longitude 3°E.

Because of its economic importance, herring has been monitored in the North Sea since 1946. The stock size for different age classes was estimated by Virtual Population Analysis based on samples of trawled herring, supported since 1968 by an international young fish survey<sup>10</sup>. The values presented here correspond to numbers of young herring entering their second calendar year on 1 January 1955 to 1987 in the northern North Sea (between latitudes 53°30'N and 62°N).

The breeding biology of the kittiwake, a pelagic seabird, has been studied since 1954 at a colony on the northeast coast of England<sup>3</sup>. We present here the annual means for laying date, clutch size and number of chicks fledged per pair from 1955 onwards. The means relate to experienced females only, to remove the effect of varying proportions of first-time breeders<sup>11</sup>, and are based on at least 24 nests except in 1955 (14 nests).

The daily weather patterns over the British Isles were classified according to the system of Lamb<sup>12</sup>. We present here the annual variations in frequency (days per calendar year) of westerly weather from 1955 to 1987.

The phytoplankton, zooplankton, herring, kittiwake breeding variables and westerly weather showed a very similar pattern in their long-term trends (Fig. 1). All started high in the late 1950s, declined at a similar rate of about 0.1 s.d. per year, reached a synchronous trough around 1979/1980, then showed a marked recovery after 1980.

The remarkable parallelism in long-term trends across four trophic levels and weather was examined more closely by means of spectral and cross-spectral analyses. The long-term trends appeared clearly in the spectra of all six time-series (Fig. 2), as high values at the lowest frequencies, that is at wavelengths greater than the length of the series. The similarity in long-term trends between the series was confirmed by consistently high

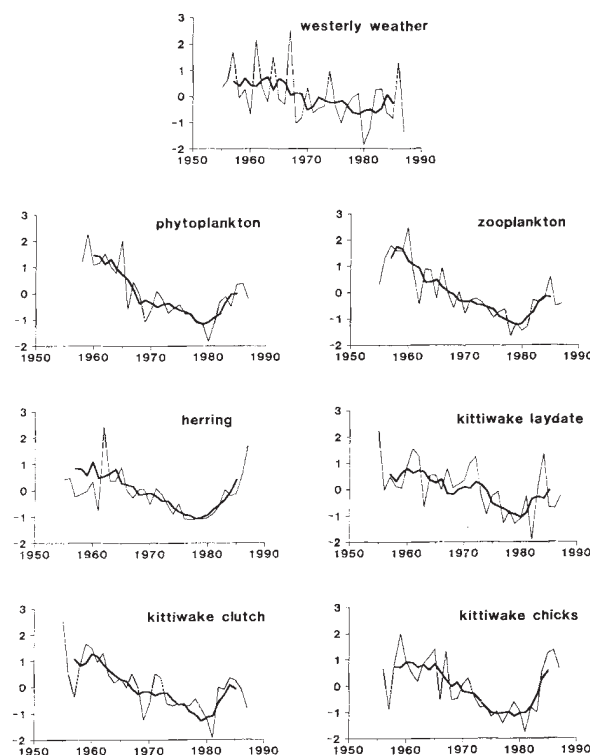


FIG. 1 Standardized (zero mean, unit variance) time-series and 5-yr running means for abundances of phytoplankton, zooplankton and herring, for kittiwake laying date, clutch size and chick production, and for frequency of westerly weather, from 1955 to 1987. Ordinates in s.d. units. In absolute terms, 5-yr running means for frequency of westerly weather ranged from 60 to 82 days  $\text{yr}^{-1}$ , corresponding to a maximum decrease of 27%.



coherences between pairs of time-series at low frequencies (Table 1, Fig. 3): averaged low-frequency coherences exceeded 0.85 for all pairs.

Another feature common to the spectra of weather and all marine trophic levels (though absent from those of kittiwake clutch size and chick production) was the secondary peak arising between frequencies 0.24 and 0.36, that is at a period of roughly 3–4 yr (Fig. 2). This peak, highest in westerly weather, was again very prominent in the coherence spectra of westerly weather against the four marine trophic levels as well as in the coherence spectra among plankton and fish (Fig. 3).

It is tempting to ascribe the similarity in patterns of annual variation between plankton, fish and bird to straight causal relationships up the food chain, the base of the chain being driven by weather (although the ability of food-chain models to predict such patterns is not necessarily trivial<sup>13,14</sup>). Alternatively, each trophic level could simply be driven directly by weather. In the first case, the response at one level would be lagged with respect to the one below it to an extent related to the internal dynamics of each level<sup>15</sup>; in the second, no such hierarchy would be expected. The phase spectra in Fig. 3 exhibited no clear pattern, however, and simulations showed that phase spectra based on 'short' series of 33 points were too inaccurate to detect presence or absence of a 1-yr lag at long wavelengths or at the 3–4-yr periodicity. It would therefore be unwise to rely on the phase spectra to identify or reject reasons for the similarities in trends between series.

Other lines of evidence indicate that any mechanisms producing the similar long-term trends are likely to be considerably more complicated than straightforward trophic interactions or direct climatic forcing. Annual variations in climate and phytoplankton certainly seem linked<sup>2,4,7</sup>, but the long-term trend in zooplankton seems to originate in winter<sup>9,16</sup> and is associated with the major current systems<sup>17</sup>. On the 3–4-yr time scale, variations in zooplankton abundance have been related to cli-

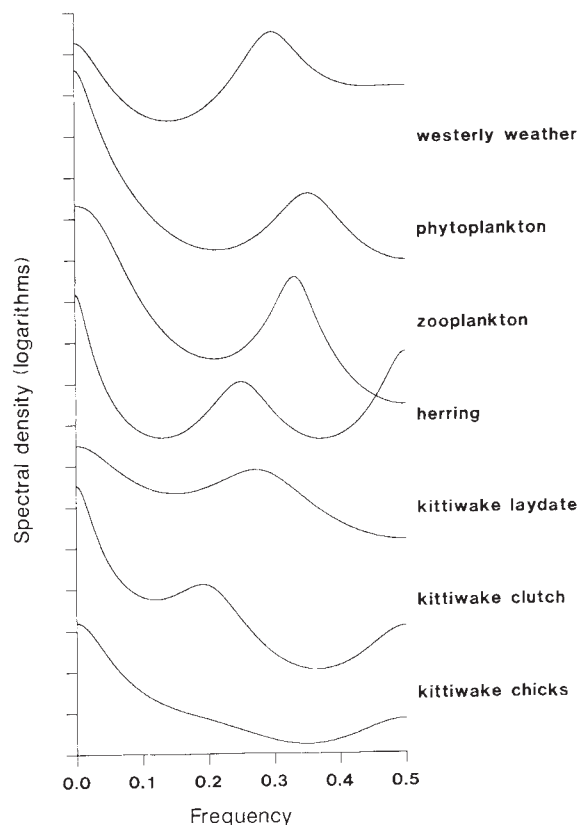


FIG. 2 Spectral density curves (logarithms) versus frequency ( $\text{yr}^{-1}$ ) for time-series of westerly weather, phytoplankton, zooplankton, herring, kittiwake laying date, kittiwake clutch size and kittiwake chick production. Spectra calculated according to Barrowdale and Erickson<sup>30</sup> with filter length of 4.

FIG. 3 Coherence and phase spectra for (left) westerly weather paired with each of phytoplankton, zooplankton, herring and kittiwake laying date, and for (right) each trophic level paired with the level above it (phytoplankton  $\times$  zooplankton, zooplankton  $\times$  herring, herring  $\times$  kittiwake laying date, herring  $\times$  kittiwake clutch size and herring  $\times$  kittiwake chick production). Spectra calculated according to Strand<sup>29</sup> with filter length of 4. Dotted line represents coherence level to exceed for significance at  $P < 0.05$ , obtained for (left) from coherence spectra of 1,000 pairs comprising westerly weather and one randomly generated time-series, for (right) from those of 1,000 pairs made up of two randomly generated time-series. Significance of phase cannot be assessed, as randomisations uniformly distributed between  $-\pi$  and  $\pi$ .

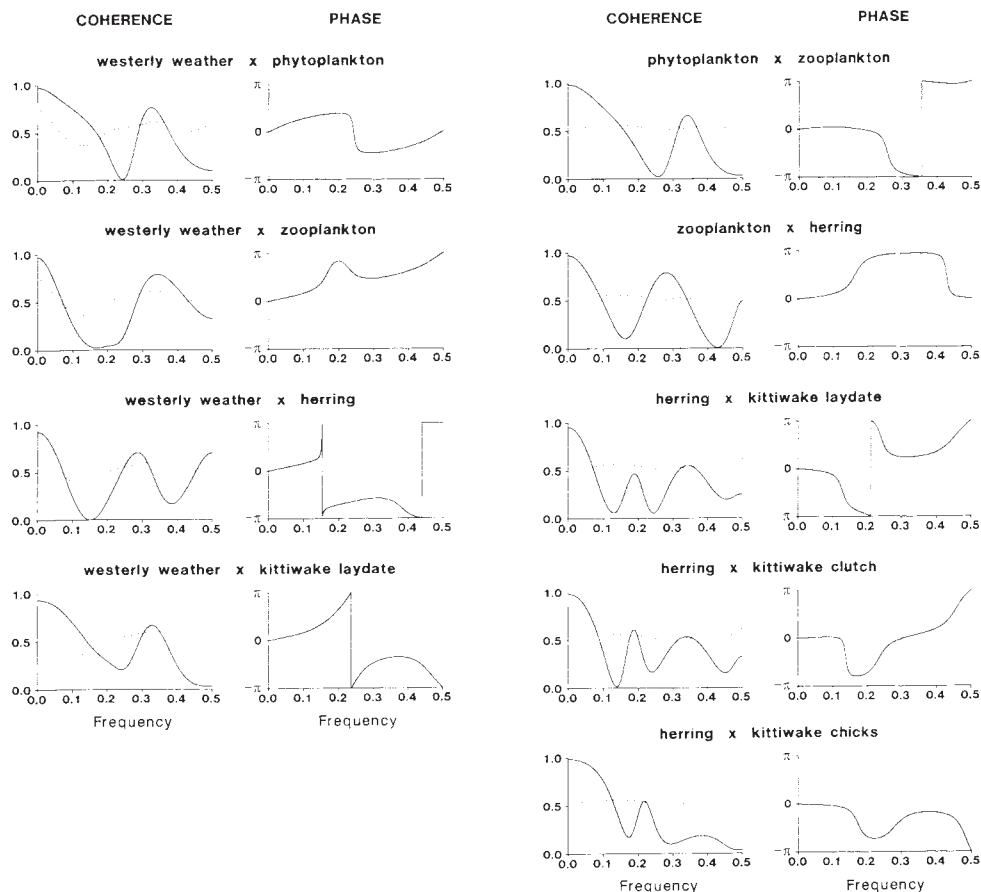


TABLE 1 Mean coherences at long wavelengths for all pairs of time-series in Fig. 1

|                       | Phyto-plankton | Zoo-plankton | Herring | Kittiwake laying date | Kittiwake clutch size | Kittiwake chicks |
|-----------------------|----------------|--------------|---------|-----------------------|-----------------------|------------------|
| Westerly weather      | 0.96           | 0.94         | 0.90    | 0.93                  | 0.96                  | 0.92             |
| Phytoplankton         |                | 0.98         | 0.98    | 0.92                  | 0.99                  | 0.93             |
| Zooplankton           |                |              | 0.95    | 0.89                  | 0.98                  | 0.85             |
| Herring               |                |              |         | 0.93                  | 0.97                  | 0.98             |
| Kittiwake laying date |                |              |         |                       | 0.95                  | 0.88             |
| Kittiwake clutch size |                |              |         |                       |                       | 0.91             |

Coherences calculated according to Strand<sup>29</sup> with filter length of 4. For each pair, value is average of coherences at frequencies of 0.005, 0.01, 0.015, 0.02 and 0.025 yr<sup>-1</sup>. All values highly significant ( $P < 0.002$  in worst case) by comparison with equivalent mean low-frequency coherences calculated from 5,000 pairs of randomly generated time-series.

mate-driven hydrological processes<sup>18</sup>. Phytoplankton may mediate between such processes and zooplankton<sup>7</sup>, but, because of nutrient recycling, the relationship could involve feedback<sup>9</sup>.

In marine fish, several studies have linked recruitment to environmental factors<sup>19–21</sup>. For herring, the situation is complicated by overfishing which is thought to have acted through the stock-recruitment relationship<sup>22</sup> to reduce stocks<sup>1</sup> until the 1977–1982 fishing ban. However, correlation between Ricker residuals and frequency of westerly weather ( $r_{31} = 0.530$ ,  $P < 0.01$ ) implied that, after removing the effect of overfishing, recruitment variability was linked to climate. The link may be through larval transport from spawning to nursery grounds<sup>23,24</sup>, as circulation in the North Sea is predominantly wind-driven<sup>25</sup>, or through other processes such as vertical mixing and the timing of stratification<sup>18</sup>. Ricker residuals and zooplankton abundance were uncorrelated, so any trophic interaction between fish and plankton was likely to be indirect and climate-driven.

The trends in kittiwake breeding variables, so similar to those in the other time-series, may reflect the predator-prey relationship between bird and fish. Indeed, food resources are an important determinant of avian breeding performance<sup>26,27</sup>, and herring constitutes an important prey item for kittiwakes before breeding (no equivalent data-series exists for *Ammodytes*, the main prey during breeding)<sup>3</sup>. Timing of breeding in seabirds has also been related to weather<sup>28</sup>, and kittiwake laying date showed coherence with weather at both long wavelengths and the 3–4-yr periodicity. Kittiwake laying date explained 47% of variation in clutch size, and the latter 47% of variation in chicks fledged, but neither relationship completely removed the long-term trends. The trends may therefore be produced by a combination of trending climate and prey.

The reasons for the parallel trends at long wavelengths and at the 3–4-yr periodicity between these series are by necessity speculative. A challenging approach which might shed light on them is modelling. Although many types of food-web model exist<sup>13–15</sup>, the results in Figs 1–3 are likely to limit the range of models capable of describing the system. Modelling will reveal the true extent of our knowledge of marine food chains, and our ability to use them to interpret and recognize signs of global climatic change. One thing is clear: the signal of weather is so strong that it is evident at all trophic levels of the marine ecosystem. □

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ACKNOWLEDGEMENTS. We thank R. S. Bailey for help with the herring data, and G. R. Potts and P. Tyler for commenting on the manuscript. We also thank D. H. Cushing and J. J. D. Greenwood for constructive criticisms.

## Overall mortality and cancer mortality around French nuclear sites

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**HIGHER** than expected mortality from leukaemia has been observed in the population under age 25 living around Sellafield and Dounreay, nuclear reprocessing plants in the United Kingdom<sup>1–4</sup>. We report the results of a similar study for the population residing around nuclear sites in France. The number of leukaemia deaths was 58, comparable to the 62 in control areas, and slightly less than the 67 expected from national mortality statistics. Twelve deaths due to Hodgkin's disease were observed around nuclear sites; this is about twice the number of Hodgkin's deaths observed in control areas and twice the number expected from national mortality statistics. This observation must, however, be interpreted in light of the fact that several causes of deaths were studied, increasing the play of chance.

France derived 75% of its electricity from nuclear energy in 1989, and the first nuclear unit producing electricity started operating industrially in 1962 (ref. 5), although experimental units started earlier. We have studied the main sites in operation during 1975 or before in order to have a minimum follow-up of 10 years for mortality. Figure 1 shows the six sites selected, together with the year of first operation and the nature of the activity<sup>6</sup>.

Four geographical zones were defined around each installation according to the distance from the installation: <5 km, 5–10 km, 10–13 km, and 13–16 km. For La Hague, the farthest zone bordered the densely populated suburbs of the city of Cherbourg: an extra zone corresponding to a distance of 16–21 km has been considered for this site. France is divided into 36,500 administrative and electoral units called 'communes'. The average population of a commune is 1,500, and the average area 15 km<sup>2</sup>. For each site and each zone, the 'exposed'

Received 10 July; accepted 9 August 1990.

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TABLE 1 Observed and expected number of deaths and standardized mortality ratios by cause, around nuclear sites and in control areas

| Cause of death                         | Exposed  |          |                      | Control  |          |                      |
|----------------------------------------|----------|----------|----------------------|----------|----------|----------------------|
|                                        | Observed | Expected | SMR (%) <sup>*</sup> | Observed | Expected | SMR (%) <sup>*</sup> |
| All malignancies                       | 166      | 171.7    | 97                   | 160      | 157.2    | 102                  |
| Brain (malignant)                      | 6        | 14.5     | 41 <sup>†</sup>      | 13       | 13.4     | 97                   |
| Brain (unspecified)                    | 16       | 14.2     | 113                  | 15       | 13.3     | 113                  |
| Lung                                   | 2        | 2.1      | 94                   | 5        | 1.9      | 263                  |
| Hodgkin's disease                      | 12       | 6.1      | 197 <sup>†</sup>     | 5        | 5.6      | 89                   |
| Other lymphomas                        | 16       | 15.5     | 103                  | 9        | 14.2     | 63                   |
| Myelomas                               | 0        | 0.3      | 0                    | 0        | 0.3      | 0                    |
| Leukaemias                             | 58       | 66.9     | 87                   | 62       | 61.2     | 101                  |
| Lymphoid leukaemias                    | 13       | 19.0     | 68                   | 14       | 17.4     | 80                   |
| All causes                             | 3,064    | 3,093.7  | 99                   | 2,828    | 2,772.2  | 102                  |
| Person-years in thousands <sup>‡</sup> |          | 2,892    |                      |          | 2,658    |                      |

<sup>\*</sup> Statistical methods: Standardized mortality ratios (SMRs), in the exposed population were compared to 100 and to SMRs in the exposed population by two-tailed tests assuming Poisson distribution<sup>8</sup>.

<sup>†</sup>  $P=0.02$  (two-sided test).

<sup>‡</sup> Estimation of person-years: for each commune the census population was available by sex and 5-year age groups. The three censuses enumerated the population respectively on 1 March, 20 February, and 4 March. We have estimated the populations on 1 January, by sex and 5-year age groups, under the assumption that the ratio of the census population to the 1 January population was the same in each commune and could be estimated by the ratio computed from INSEE publication for the whole of France. Yearly populations on 1 January were computed by linear interpolation between the populations on 1 January for census years, for a given sex and age group. After 1982, the 1 January population of each commune for each year was extrapolated from the 1982 1 January population, under the assumption that this population varied like the total French population of the same sex and age group for which extrapolations are published by INSEE. For each year and each commune the population at risk is the average of the population on 1 January of that year and of the next year.

communes were identified by their national code, and for each exposed commune, a 'control' commune was selected as the commune in the same 'Département' having the closest total population figure. This defines, for each site, four (five for La Hague) exposed zones according to the distance to the installation, and the same number of control zones. The average distance between control communes and installation is 53 km (range 16–133 km, s.d. 24 km).

From the Institut National de la Santé et de la Recherche Médicale, service commun 8 (E. Michel and F. Hatton, personal communication), we obtained the cause of each death that occurred in the population aged 0–24 between 1968 and 1987, by zone (the precise commune of residence corresponding to each death was not made available to us in order to maintain confidentiality over the cause of death required by French law). The underlying cause of each death was coded according to the International Classification of Diseases (eighth revision before 1979, and ninth revision after). Census data by commune were obtained from the Institut National de la Statistique et des Etudes Economiques (INSEE) for the three censuses of 1968,

1975, and 1982. The populations at risk were estimated from these data (see legend to Table 1).

Two sites started operation after 1968: Saint Laurent in January 1969 and Saint Vulbas in December 1971. Deaths occurring before 1969 and before 1972 respectively in these two sites were excluded from our study as well as the corresponding population at risk.

To test the possible existence of an increase in leukaemia mortality between age 0 and 24 around French nuclear sites, we made two comparisons. First, the observed mortality was compared to the mortality expected from national rates<sup>7</sup>. Second, in an attempt to control for possible systematic differences between death certification procedures in rural, sparsely populated areas and in the country as a whole, the mortality around nuclear sites was compared with the mortality in control communes, matched for total population and large geographical unit (Département).

Table 1 gives the observed and expected number of deaths and the standardized mortality ratio for relevant causes, and the size of the population at risk, for both exposed and control

TABLE 2 Number of person-years, observed and expected number of leukaemia deaths, and standardized mortality ratios by sex, age, type of installation and distance from nuclear installations

| Characteristics |              | Person-years in thousands | Observed | Expected | SMR (%) |
|-----------------|--------------|---------------------------|----------|----------|---------|
| Sex             | Male         | 1,486                     | 34       | 39.6     | 86      |
|                 | Female       | 1,406                     | 24       | 27.4     | 88      |
| Age             | 0–4          | 583                       | 13       | 13.9     | 93      |
|                 | 5–9          | 588                       | 12       | 17.5     | 69      |
|                 | 10–14        | 613                       | 7        | 13.6     | 52      |
|                 | 15–19        | 589                       | 16       | 12.4     | 129     |
|                 | 20–24        | 519                       | 10       | 9.6      | 105     |
| Installation    | Reprocessing | 1,576                     | 30       | 36.7     | 82      |
|                 | Other        | 1,316                     | 28       | 30.2     | 93      |
| Distance in km  | <5           | 260                       | 5        | 6.1      | 82      |
|                 | 5–9.9        | 982                       | 21       | 22.7     | 93      |
|                 | 10–12.9      | 373                       | 4        | 8.5      | 47      |
|                 | 13–15.9      | 748                       | 17       | 17.3     | 92      |
|                 | 16–21        | 530                       | 11       | 12.3     | 90      |
| Total           |              | 2,892                     | 58       | 66.9     | 87      |

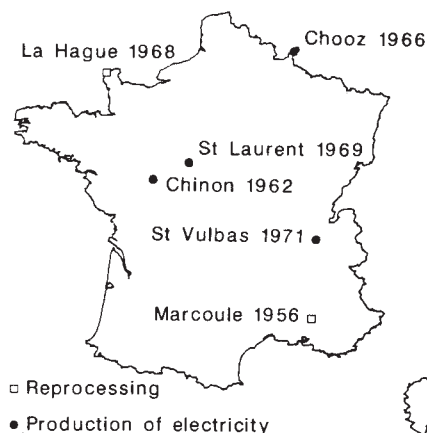


FIG. 1 Nuclear sites considered and year of first operation. The site of La Hague includes only reprocessing activities, whereas Marcoule includes both reprocessing activities and reactors. Chooz includes pressurized water reactors; the other sites where electricity is produced started operation with a graphite-gas reactor; pressurized water reactors were installed in later years.

populations. In the exposed population, the number of leukaemia deaths was 58, which is slightly less than the 66.9 deaths expected from national mortality statistics. Among the other causes considered, two significant differences (both with  $P=0.02$ , two-sided test) were observed between nuclear sites and national mortality: an excess of Hodgkin's disease, and a deficit of malignant brain tumours. After correction for the multiple tests due to the consideration of several causes of death, these results are no longer significant. No significant differences were observed when comparing the standardized mortality ratios in the exposed and control areas, but these comparisons are less powerful than the comparison of the exposed population to the nation as a whole. The smallest  $P$  values were 0.12 and 0.20 for malignant brain tumours and Hodgkin's disease respectively; the  $P$  value for leukaemia was 0.45.

Table 2 gives the number of leukaemia deaths by sex, age, type of installation (reprocessing plants versus others) and distance from installation. There is no effect of sex and age, no difference between reprocessing plants and reactors, and no trend with increasing distance from installation.

Our study shows no excess leukaemia mortality in the population aged 0–24 residing near French nuclear sites. The power

of this study is reasonable: when the reference is the general population, and with an expected number of leukaemias around installations equal to 60, the probability of detecting an increase of 50% is 95% (with a type I error of 5%), and the probability of detecting an increase of 23% is ~50% (ref. 8). When the reference is a control group of similar size, the probability of detecting an increase of 50% is ~80% (ref. 8).

Our results confirm Viel and Richardson's study of leukaemia mortality around La Hague<sup>9</sup>, which used geographical units with populations seven times larger than in our study. The excess leukaemia observed around nuclear sites in the United Kingdom is not observed around French nuclear sites, although the same methodology was used as in ref. 4.

The amount of radioactive effluent discharged might have been higher around Sellafield and Dounreay than around French installations<sup>9</sup>. The excess leukaemia observed in the United Kingdom could also be attributed to some characteristic common to Sellafield and Dounreay, but not shared by French installations, for instance a rapid increase of population leading to viral infections<sup>10</sup>, or some unknown factor shared by existing and potential nuclear sites in the United Kingdom<sup>11</sup>. □

Received 13 July; accepted 10 September 1990.

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ACKNOWLEDGEMENTS. We thank A. Auquier and R. Doll for comments on the manuscript and A. Leszczynski for secretarial assistance. This work was partly supported by a grant from La Ligue Nationale Contre le Cancer.

## Self-incompatibility in *Nicotiana alata* involves degradation of pollen rRNA

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GAMETOPHYTIC self-incompatibility is a genetically based system of cellular recognition in plants<sup>1</sup>. It prevents fertilization by pollen bearing an *S*-allele identical to either of the two *S*-alleles present in the female sporophytic tissues. Self-incompatibility in the Solanaceae has been especially well studied and several *S*-allele specific style glycoproteins identified<sup>2–6</sup>. In addition, complementary DNAs for nine style *S*-glycoproteins have been sequenced<sup>7–10</sup> and have homology with two fungal ribonucleases. Recently five *Nicotiana alata* *S*-glycoproteins were shown to be RNases (*S*-RNases)<sup>11</sup>. We now report that *S*-allele specific degradation of pollen RNA occurs *in vivo*. After incompatible, but not after compatible pollinations, pollen RNA becomes degraded. This specificity cannot be demonstrated *in vitro* using isolated *S*-RNases and pollen RNAs. Our results support a model in which the gametophytic self-incompatibility system in *N. alata* acts through a cytotoxic mechanism directed against pollen RNA.

The finding that style *S*-glycoproteins from *N. alata* are RNases<sup>11</sup> leads to the question of whether this enzymatic function has a role in expression of self-incompatibility (SI). To approach this question we obtained labelled pollen by growing plants in the presence of <sup>32</sup>P and followed the fate of labelled RNA within styles after pollination (Fig. 1a). In both compatible and incompatible crosses in *N. alata*, pollen germinates and pollen tubes grow through the stigma into the transmitting tissue

of the style. Initially, pollen tubes of the crosses are morphologically indistinguishable; the walls of the incompatible tubes gradually become thickened and growth is arrested in a distinct zone of the style about 5–10 mm below the stigma (Fig. 1b). We prepared RNA separately from stigma and style fractions of the pistil (Fig. 1b). The RNA from the style fractions contains [<sup>32</sup>P]RNA derived from pollen tubes that have grown through the style to the region where growth is inhibited. We found that the total amount of [<sup>32</sup>P]RNA recovered from the style after incompatible pollination was less than that recovered from compatible pollination (Table 1). Two factors could contribute to this effect. Either fewer pollen tubes enter the incompatible style, and/or less RNA is recovered from tubes which successfully enter the style. The experiments shown in Fig. 1 and the knowledge that germination and growth of compatible and incompatible pollen is similar in the upper part of the style, suggest that low recovery of RNA from incompatible pollen tubes is an important effect (see below). Recovery of RNA from stigmas was variable. The variation had no apparent relationship to SI (Table 1 legend), but may reflect the variability of pollen adhesion, germination and early growth between experimental pollinations.

Agarose-gel fractionation of the [<sup>32</sup>P]RNAs recovered from styles after compatible and incompatible crosses shows that the ribosomal RNAs are intact in the styles of compatible crosses but degraded in incompatible crosses (Fig. 1c). As less [<sup>32</sup>P]RNA is recovered from styles of the incompatible crosses, parallel analyses based on equal total RNA loading and on loading of equal [<sup>32</sup>P]RNA were undertaken. The result was essentially the same for both approaches (Fig. 1c). In nine of ten separate experiments, there was no obvious degradation of [<sup>32</sup>P]RNA in the stigma fraction from either compatible or incompatible crosses.

The [<sup>32</sup>P]RNA from the style fraction of incompatible crosses appears as a 'smear' in which discrete species in the range 1,750–400 nucleotides can be recognized (Fig. 1c). These



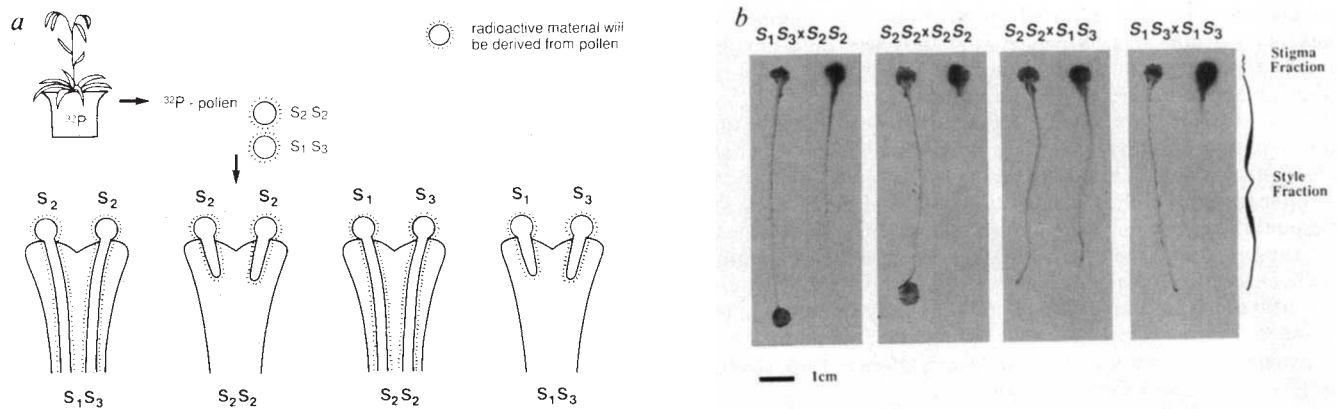
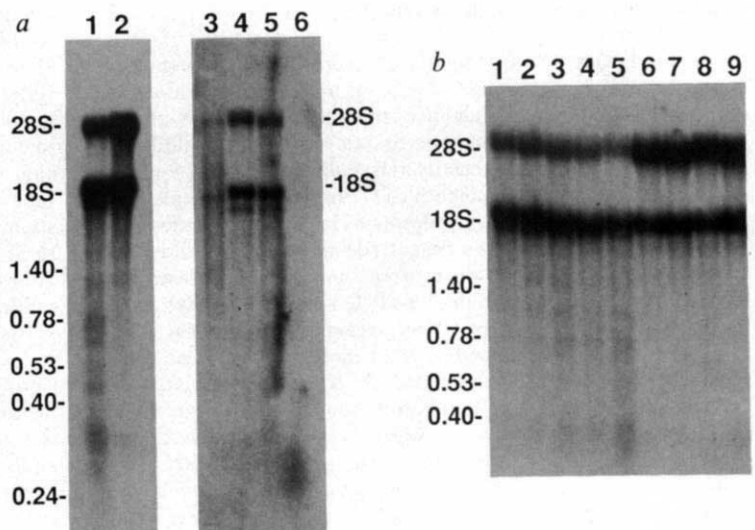


FIG. 1 S-allele-specific degradation of pollen RNA within the style. *a*, Experimental design. *N. alata* plants were grown with [ $^{32}\text{P}$ ]orthophosphate to obtain [ $^{32}\text{P}$ ]pollen which was applied to pistils of unlabelled flowers. Reciprocal crosses were performed involving the *S*-genotypes shown. *b*, Autoradiography of styles 48 h after pollination. Pistils were pressed onto nitrocellulose for autoradiography. The shape of the stigmas and ovaries was distorted in this procedure: ovaries were often detached (for example  $S_2S_2 \times S_1S_3$  and  $S_1S_3 \times S_1S_3$  crosses). Both dried pistils (left) and autoradiograms (right) are shown for each of four crosses. In compatible crosses, the tubes have grown 5–6 cm, whereas in the incompatible crosses the tubes are about 1.0 cm long. The upper 1–1.5 mm, which includes the stigma (stigma fraction) was excised. The style fraction included the zone between the lower part of the stigma and the upper part of the ovary (style fraction). *c*, Analysis of RNA prepared in experiments 1 and 2 of Table 1. RNA was fractionated in a 2% agarose-formaldehyde gel<sup>19</sup>, blotted on to a nylon membrane and autoradiographed at  $-70^\circ\text{C}$  with an intensifying screen for 7 days. The stigma and style fractions were treated separately. The gel lanes containing the style fraction RNAs were loaded both by equal [ $^{32}\text{P}$ ] counts and by equal amounts of RNA as shown. [ $^{32}\text{P}$ ]RNA from the style fractions derived from the compatible crosses ( $S_1S_3 \times S_2S_2$ ;  $S_2S_2 \times S_1S_3$ ) consists largely of intact 28S and 18S rRNA. In contrast, [ $^{32}\text{P}$ ]RNA from the style fractions derived from the self-incompatible crosses ( $S_2S_2 \times S_2S_2$ ;  $S_1S_3 \times S_1S_3$ ) shows degradation of the 28S and 18S rRNA as well as accumulation of lower- $M_r$  species. This pattern is seen both in lanes loaded for equal [ $^{32}\text{P}$ ] counts or equal amounts of RNA. [ $^{32}\text{P}$ ]RNA from the stigma fractions shows intact 28S and 18S RNA from all crosses. Tissue and RNA preparation were as described in Table 1.

FIG. 2 Analysis of [ $^{32}\text{P}$ ]RNA from an incompatible pollination ( $S_1S_3 \times S_1S_3$ ). Lanes 1, 2: poly(A)<sup>+</sup> fractions. Lane 1, style RNAs which include a range of low- $M_r$  species; lane 2, stigma RNAs which are essentially undegraded; lanes 3, 4, 5, 6: comparison of RNA fractions from styles; lane 3, poly(A)<sup>+</sup> RNA; lane 4, poly(A)<sup>-</sup> RNA; lane 5, total LiCl-precipitated RNA; lane 6, LiCl-soluble material. RNAs in lane 1 and 4 are the same sample but different loadings. The low- $M_r$  bands are not enriched in the poly(A)<sup>+</sup> fraction or the LiCl-soluble material. *b*, Progressive degradation of [ $^{32}\text{P}$ ]RNA species after incompatible pollination. Lanes 1–5, [ $^{32}\text{P}$ ]RNAs from styles collected 12, 18, 24, 36, 48 h after incompatible pollination ( $S_1S_3 \times S_1S_3$ ); lane 6, stigma [ $^{32}\text{P}$ ]RNA 48 h after incompatible pollination ( $S_1S_3 \times S_1S_3$ ); lane 7, style [ $^{32}\text{P}$ ]RNA 48 h after compatible pollination ( $S_2S_2 \times S_1S_3$ ); lane 8, stigma [ $^{32}\text{P}$ ]RNA 48 h after compatible pollination ( $S_2S_2 \times S_1S_3$ ); lane 9, control extraction in which [ $^{32}\text{P}$ ]pollen ( $S_1S_3$ ) was mixed with unlabelled styles and the RNA immediately extracted (the stigmas were not removed in this experiment). The low- $M_r$  material accumulates progressively during the 48 h period after incompatible pollination. This low- $M_r$  material is not seen in stigma fractions; the control experiment indicates that it is not an artefact of extraction. The size of the markers is given in kilobases.

**METHODS.** Tissue and RNA preparation are described in Table 1. RNAs were applied to oligo(dT)-cellulose in 1 M NaCl, 10 mM Tris, pH 8, 1 mM EDTA, poly(A)<sup>+</sup> RNA was eluted with water. RNAs soluble in 4 M LiCl (see methods described in Table 1 legend) were recovered by precipitation from ethanol. In both *a* and *b*, RNAs were separated in 2% agarose-formaldehyde gels<sup>19</sup>



blotted onto a nylon membrane and [ $^{32}\text{P}$ ]RNAs visualized by autoradiography (17 days in *a*, 9 days in *b*) as described in Fig. 1. Unlabelled poly(A)<sup>+</sup> RNA from  $S_1S_3$  pollen was added to adjust the specific activity of samples to the same level.

products of low relative molecular mass ( $M_r$ ) were recovered in the poly(A)<sup>-</sup> fraction (Fig. 2a), and accumulate progressively after pollination (Fig. 2b). Neither the poly(A)<sup>+</sup> fraction nor the LiCl supernatant of incompatible crosses contained these low- $M_r$  species (Fig. 2a). These observations indicate that the low- $M_r$  species are degradation products of rRNA as transfer RNA would be found in the LiCl supernatant and messenger RNA in the poly(A)<sup>+</sup> fraction.

These experiments suggest that expression of SI is mediated by degradation of pollen rRNA of incompatible pollen tubes during their growth within the style. This suggestion is supported by the *S*-allele specific pattern of rRNA degradation, the onset and time course of degradation which parallels that of tube growth inhibition, and the knowledge that the *S*-glycoproteins are RNases.

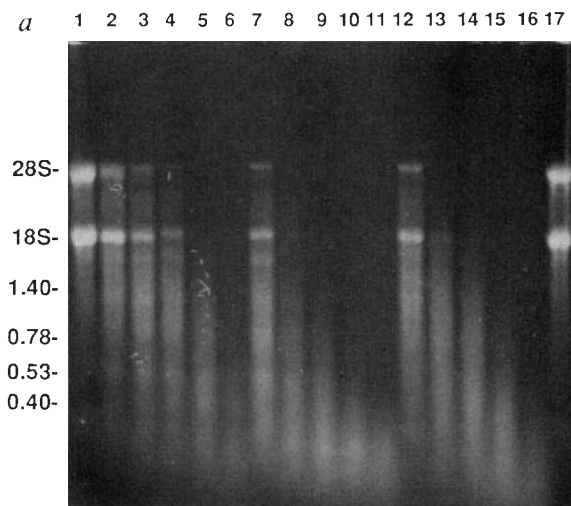
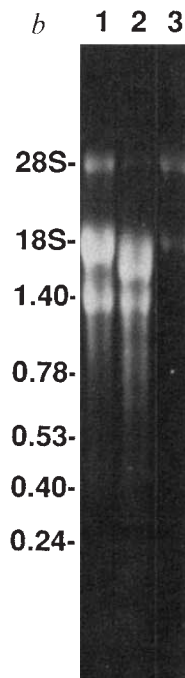


FIG. 3 Action of isolated style *S*-glycoproteins on pollen RNA *in vitro*. *a*, Digestion of pollen RNA. Lane 1, undigested  $S_6S_6$  pollen RNA; lanes 2–16,  $S_6S_6$  pollen RNA digested for 5, 10, 15, 30 and 60 min with 0.0026 U  $S_2$ -RNase (lanes 2–6), 0.0053 U  $S_6$ -RNase (lanes 7–11) or 0.0048 U RNase A (Sigma, R-4875; lanes 12–16); lane 17,  $S_6S_6$  pollen RNA incubated for 60 min with no added RNase. The  $S_6S_6$  pollen RNA is progressively digested by RNase in each case and the pattern of digestion is similar in each case. Pollen RNA and isolated *S*-RNases from other *S*-genotypes gave similar results. Digestions containing the indicated amount of RNase were in 0.4 ml 0.01 M potassium phosphate, pH 7, 0.005 M KCl, 0.4 mg ml<sup>-1</sup> RNA. The RNA substrate was prepared from mature  $S_6S_6$  pollen as described in the methods for Table 1. RNase activities were determined by a perchloric acid solubilization method essentially as described<sup>11</sup> (1 U = 1 A<sub>260</sub> min<sup>-1</sup> ml<sup>-1</sup> (ref. 20)). Digestions at 37 °C were terminated by diluting an aliquot (50 µl) into 450 µl of 7 M urea, 0.1 M Tris pH 8, 0.01 M EDTA, 1% SDS, immediately extracting with phenol-chloroform-isoamyl alcohol. Digested RNAs were precipitated, suspended, in water, denatured, separated in a 1.75% agarose-formaldehyde gel<sup>19</sup> and stained with 0.5 µg ml<sup>-1</sup> ethidium bromide. *b*, Digestion of ribosomes by  $S_2$ -RNase *in vitro*. Lane 1, wheat germ translation extract (Promega) incubated as recommended, except that RNase inhibitor, mRNA and labelled amino acids were omitted; lane 2, incubation mixture as for lane 1, to which 0.2 U ml<sup>-1</sup>  $S_2$ -RNase was added. Incubations were extracted and separated in a 2% agarose formaldehyde gel as described above; lane 3, 28S and 18S rRNA. The depleted 28S band and accompanying material running ahead of the 18S band indicates digestion of rRNA in ribosomes by  $S_2$ -RNase. The size of the markers is given in kilobases.



The *S*-allele specific degradation of pollen rRNA associated with self-pollination *in vivo* is not observed in *in vitro* experiments. For example, RNA from pollen genotype  $S_6S_6$  was equally well digested *in vitro* with purified style *S*-RNases from genotypes  $S_2S_2$  and  $S_6S_6$  as well as RNase A (Fig. 3a). Thus the pollen RNAs are not specifically tailored for the corresponding style *S*-RNase. Neither are the *S*-RNases highly site specific; digestion of RNA substrates with each of the *S*-RNases gives a similar pattern (Fig. 3a), which is in turn similar to that obtained from the action of a nonspecific RNase such as RNase A, and is distinct from that expected of a site-specific RNase such as the action of  $\alpha$ -sarcin on ribosomes<sup>12</sup>. The rRNAs of ribosomes in wheat germ translation extracts are also substrates for  $S_2$ -RNases (Fig. 3b).

Loss of rRNA in pollen tubes would lead to arrest of pollen tube protein synthesis. In germinated *Tradescantia* pollen<sup>13</sup>, there is apparently no transcription of ribosomal genes and our experiments show that this is also the case for *N. alata* pollen germinated *in vitro*. Incorporation of [<sup>32</sup>P]orthophosphate is primarily into LiCl-soluble RNA and into poly(A)<sup>+</sup> RNA. There is little or no incorporation into rRNA (B.A.McC., unpublished observations). These results are consistent with observations that *in vitro* pollen tube growth is inhibited by cycloheximide but not actinomycin D<sup>13</sup>. This peculiar lack of rRNA synthesis in pollen makes it extremely vulnerable to depletion of its stored rRNA; in short, degradation of pollen rRNA would lead to depletion of the protein biosynthetic apparatus and eventual arrest of tube growth. The experiments described are consistent with this mechanism for arrest of pollen tube growth in incompatible matings. They do not, however, exclude the possibility that degradation of rRNA is a secondary consequence of some other inhibitory mechanism.

Targeting of rRNA is a strategy for selective toxicity in several other systems<sup>14</sup>. The bacterial cytotoxins, colicin E3 and colicin Df13, are RNases specific for 16S RNA<sup>15</sup>. Many plants contain

TABLE 1 Recovery of pollen RNA from *N. alata* styles after compatible and incompatible pollinations

| Expt No. | Cross                               | No. of flowers | [ <sup>32</sup> P]RNA recovered* | Ratio of [ <sup>32</sup> P]RNA† |
|----------|-------------------------------------|----------------|----------------------------------|---------------------------------|
| 1        | Compatible $S_3S_3 \times S_2S_2$   | 32             | 161                              | 0.30                            |
|          | Incompatible $S_2S_2 \times S_3S_3$ | 30             | 49                               |                                 |
| 2        | Compatible $S_2S_2 \times S_1S_3$   | 32             | 165                              | 0.26                            |
|          | Incompatible $S_1S_3 \times S_2S_2$ | 30             | 43                               |                                 |
| 3        | Compatible $S_6S_6 \times S_1S_3$   | 22             | 88                               | 0.20                            |
|          | Incompatible $S_1S_3 \times S_6S_6$ | 24             | 18                               |                                 |
| 4        | Compatible $S_6S_6 \times S_2S_2$   | 23             | 82                               | 0.37                            |
|          | Incompatible $S_2S_2 \times S_6S_6$ | 23             | 30                               |                                 |

\* As c.p.m. per style recovered from extracts of pollinated styles.

† Incompatible c.p.m. per style to compatible c.p.m. per style in extracts of styles after compatible and incompatible pollinations.

Compatible and incompatible crosses with <sup>32</sup>P-labelled pollen were performed, and the styles collected 48 h later. The stigmas (upper 1–1.5 mm of pistil) and styles were treated separately. Consistently more <sup>32</sup>P was recovered from styles of compatible crosses than from incompatible crosses. The corresponding data for <sup>32</sup>P recovered from stigmas (c.p.m. per stigma) of the crosses are: Experiment 1: 76, 232; experiment 2: 148, 45; experiment 3: 21, 84; experiment 4: 84, 119. These experiments were repeated on six separate occasions with similar results.

Genetically defined *N. alata* stocks were grown and maintained as previously described<sup>9</sup>. Periodically, sufficient <sup>32</sup>P (Amersham, PBS.11) was added to maintain the total activity of each plant at 1–3 mCi (37–111 TBq) during the peak flowering period. Pollen from fully opened anthers was either used fresh or stored for 1–2 days at –70 °C. Control pollinations always showed full seed set in compatible crosses and no seed set in incompatible pollinations. Forty-eight hours after pollination, flowers were collected, stigmas and styles separated, immediately frozen in liquid nitrogen, and stored at –70 °C for analysis. Frozen tissues (50–450 mg) were homogenized in 5 ml of 7 M urea, 0.1 M Tris-HCl pH 8, 0.01 M EDTA, 1% (w/v) SDS, 0.1 M 2-mercaptoethanol plus 5 ml of phenol-chloroform-isoamyl alcohol for 3 min at high speed with a Polytron. After centrifugation the aqueous phase was recovered and sequentially extracted with phenol-chloroform-isoamyl alcohol (25:24:1) and chloroform. Crude nucleic acids were precipitated from 2-propanol as the ammonium salt, pelleted, and resuspended in 3 ml of water to which 3 ml of 8 M LiCl were added. After 0.5–2 h on ice, RNAs were pelleted, resuspended in water, and precipitated from ethanol as ammonium salts. Radioactivity of pelleted RNA was determined by Cerenkov counting in 1.5-ml plastic centrifuge tubes with a liquid scintillation counter.



ribosome-inactivating proteins<sup>16</sup> which act by hydrolysing a specific *N*-glycosidic bond in 28S RNA<sup>17</sup>. Although extracellular RNA and RNases have been suggested as components of a regulatory system in animal tissues<sup>18</sup>, it is unlikely that an extracellular substrate is involved in the SI system in view of the dramatic degradation of rRNA in the pollen tubes.

A major unresolved question is the nature of allelic specificity in SI. It is unlikely that specificity resides in the substrate for the *S*-RNases as they degrade RNA from many sources. Neither do *S*-RNases behave in a way analogous to the DNA restriction endonucleases as there is no similar base specificity. Further, we have not been able to demonstrate any *S*-allele specific inhibition of *S*-RNase by pollen tube extracts (B.A.McC., unpublished observations), which makes the possibility of specificity being expressed in this way unlikely.

One hypothesis that we are testing is that the *S*-allele specificity of interaction between pollen tube and style resides in the mechanism by which the *S*-RNase gain access to the cytoplasmic compartment of the pollen tube. □

Received 12 June; accepted 24 August 1990.

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ACKNOWLEDGEMENTS. We thank A. Bacic, V. Haring, S. Read, G. McFadden and S.-L. Mau for discussion, I. Bönig for assistance with photography, and B. McGinness and S. Mau for maintaining plants in the glasshouse and collecting material. J.E.G. was supported by a Fellowship from the Royal Society of London.

## Swimbladder acoustic pressure transduction initiates Mauthner-mediated escape

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**THE bilateral Mauthner neurons initiate the characteristic 'C-start' escape of fishes when they respond to underwater sounds accompanying attacks by predators<sup>3-6</sup>. Either of the two sound components, particle motion or pressure change can initiate Mauthner-mediated escape. Particle motion can be detected by inner ear sensory receptors and used for locating a sound source<sup>7</sup>, but sound pressure requires a transduction mechanism to stimulate the same receptors<sup>8-10</sup>. For fishes, sound pressure is a nondirectional scalar signal that is transduced by the swimbladder<sup>11,12</sup>. It might be assumed that particle motion, because of its directional nature, triggers Mauthner-initiated predator evasion<sup>2</sup>, but we demonstrate here that for acoustically mediated escape, sound pressure is the salient stimulus for activating the Mauthner neurons**

**in fishes with swimbladders. This implies that displacement determines which of the two Mauthner neurons is activated, so that the fish turns away from the direction of the predatory attack.**

Sounds produced in air above a restrained goldfish (Fig. 1) cause short-latency, auditory-evoked postsynaptic potentials (p.s.ps) in the Mauthner (M-) cells (Fig. 2a; arrow, trace 1). Such p.s.ps are significantly decreased when the swimbladder is punctured with a syringe needle and are eliminated by creating a vacuum in the swimbladder (Fig. 2a, trace 2). When the bladder is re-inflated, a partial recovery is recorded (Fig. 2a, trace 3). We repeated 23 reversals sequentially while recording from the M-axons in four fish. These findings parallel hearing changes due to swimbladder manipulations<sup>13,14</sup> and demonstrate the crucial role of this organ in acoustic activation of the M-cells.

This experiment does not show that acoustic pressure can fire the M-cell to produce a behavioural response. Most of the sound

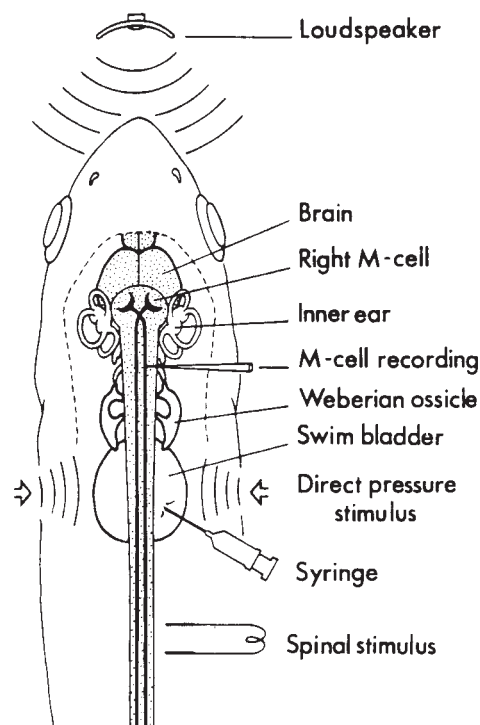


FIG. 1 Schematic diagram of the experimental apparatus.

**METHODS.** Six adult goldfish were each supported in a V-shaped structure such that only the portion of the body below the lateral line was underwater. Fish were kept moist while anesthetized and respired continuously through the mouth with chilled water. Airborne audio pulses of 5.7 Pascals were generated by a loudspeaker 65 cm anterior to the fish. Fourier analysis of this stimulus had peak frequencies of 0.4, 1.8, and 2.9 kHz. The latter frequency is one for which behavioural pressure detection is the same whether the sound is propagated through air or water<sup>30</sup>. Direct pressure stimuli were delivered bilaterally to the body surface dorsolateral to the anterior chamber of the swimbladder with inverted syringe needles mounted in two micropipette holders and driven by nitrogen gas pulsed at 20 p.s.i. for 6 ms. The pressure component of either stimulus was transduced by the swimbladder and, in goldfish and other otophysan fishes, transmitted through Weberian ossicles and perilymphatic fluids to both saccular maculae. Each ear sends afferents to the M-soma on its respective side, and acoustic pressure-evoked p.s.ps generated at both somata were recorded intracellularly from either M-axon between the vagal lobes. To facilitate identification, M-axons were antidromically stimulated with bipolar spinal stimuli of 0.03-ms duration, 4-5 V, and at 1-2 pulses/s. M-axon recordings were made with 8-10 M $\Omega$  potassium acetate micropipette electrodes. To release swimbladder pressure, a syringe needle was inserted through the dorsolateral musculature away from the posterior lateral line nerve. Initial loss of swimbladder pressure often produced a startle response so, before puncturing the bladder, the intracellular electrode was withdrawn to prevent M-axon damage from the animal's movement. Loss of the p.s.p. due to current shunting from the penetration was controlled for by repenetrating the same M-axon about 250  $\mu$ m anterior to the previous site and by penetrating the intact contralateral axon.

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stimulus is reflected owing to the impedance mismatch between air and the body of the fish. To circumvent this problem, pressure pulses were applied directly to the body (Fig. 1). This stimulus also resulted in a rapid onset p.s.p. (Fig. 2*b*; arrow, trace 1), which was eliminated by swimbladder puncture and vacuum (Fig. 2*b*, trace 2). Moreover, higher-intensity pressure-evoked p.s.ps could initiate an M-cell action potential (Fig. 2*c*; arrow, trace 1). The resulting startle response caused a loss of the intracellular electrode penetration (dot, trace 1). This experiment demonstrates the neuroethological relevance of sound pressure transduction for eliciting escape.

In earlier studies involving M-cell acoustic or vibrational stimulation<sup>1,2,15</sup>, sound pressure and displacement could not be separated. Our direct pressure stimulus generated head displacements of  $0.26\text{ }\mu\text{m}$ . This is 2,600 times the displacement threshold for goldfish acoustic fibres<sup>7</sup>. But after swimbladder deflation, neither this stimulus nor the airborne auditory stimulus elicited a measurable depolarization. This rules out M-cell activation by acoustic particle motion.

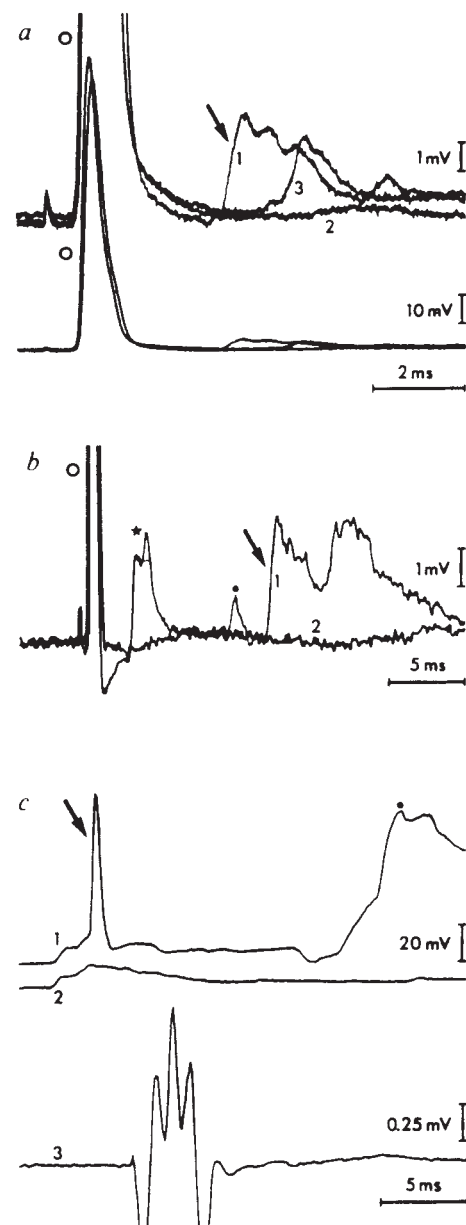
Our study provides neurophysiological support for the prediction made from behavioural analyses by Blaxter and colleagues<sup>3,16</sup> that pressure elicits 'Mauthner-type' responses in adult herrings. In larval herring<sup>17</sup> and larval zebra fish<sup>18</sup>, low-

frequency particle motion may activate the M-cell through the lateral line system. In the adult, activation by motion-sensitive modalities such as lateral line is unlikely because of the absence of Mauthner depolarization in response to the large displacement of our stimulus. This is consistent with a previous demonstration<sup>19</sup> that both natural and electrical stimulation of the lateral line failed to fire the goldfish M-cell. Therefore, in response to the sounds accompanying predatory attack, sound pressure transduction is the salient stimulus for eliciting M-initiated escape responses in adult fishes with swimbladders.

This presents a paradox. The direction of the ensuing escape response is paramount to survival. Why initiate the behaviour with acoustic pressure when directional information is inherent in particle motion? The answer may be that, relative to acoustic pressure intensity, particle motion attenuates faster with distance by roughly an order of magnitude<sup>12</sup>. Furthermore, the gas-filled swimbladder serves to enhance detection of<sup>12,20</sup> and amplify<sup>11,21</sup> changes in sound. Thus, fish with swimbladders have increased their effective acoustic range for escape initiation by adapting pressure transduction for initiating predator evasion.

Detection of particle motion probably encodes stimulus direction and modulates which of the two M-cells is activated. This is particularly relevant in light of M-cell neurophysiology and

FIG. 2 Intra-axonal recordings of acoustically evoked p.s.ps. Antidromically evoked M-cell spikes (open circles) in (a) and (b) are used to identify the axons. *a*, Upper traces, three superimposed, high-gain p.s.p. recordings from an M-axon in response to airborne auditory pulses. As measured with a hydrophone,  $\sim 2\text{ ms}$  were required for the airborne sound pulse to reach the fish from the speaker, yielding  $1.5\text{ ms}$  latency from stimulus arrival to p.s.p. onset in these experiments. Trace 1, Audio-evoked p.s.p. (arrow) before swimbladder puncture. Trace 2, Swimbladder puncture followed by a slight vacuum eliminated the p.s.p. Trace 3, Partial restoration of the audio-evoked p.s.p. after reinflation. Lower traces are low-gain display of upper traces. Fifteen reversals were repeated sequentially while recording from the first M-axon penetrated in 4 fish. To control for axonal damage, 8 additional reversals were demonstrated in the contralateral M-axon of two of these fish. *b*, Two superimposed, high-gain records of audio- and direct pressure-evoked p.s.ps. Trace 1, The first audio-evoked p.s.p. (asterisk) is the same as that recorded in *a*. A smaller audio-evoked p.s.p. (dot) occurs at longer latency. A direct pressure-evoked p.s.p. (arrow) appears at even longer ( $7\text{ ms}$ ) latencies owing to the mechanics of the pressure apparatus. Trace 2, All p.s.ps were eliminated by swimbladder puncture and vacuum. In pilot work even a slight vacuum of the coelomic cavity that did not puncture the swimbladder decreased the p.s.p., but no amount of coelomic vacuum eliminated it. Thus, p.s.p. changes probably reflect a loss of swimbladder transduction mechanics and not a disruption of inner ear function. *c*, Pressure-evoked, orthodromic activation of the M-cell, with resultant startle responses, were demonstrated in two other fish. Traces 1 and 2 have been offset vertically to illustrate the p.s.p. Trace 1, A high-intensity pressure pulse was used to elicit an orthodromic M-cell action potential (arrow). Trace 2, The previous stimulus pulse generated only a p.s.p. Trace 3, Ipsilateral epaxial-recorded electromyogram of resultant M-cell activation. The consequent movement caused the loss of the recording electrode penetration (trace 1, dot).





predicts the ethological relevance of Mauthner-associated neural networks<sup>31</sup>. The 'commissural inhibitory network' normally suppresses both M-cells at low acoustic intensities and prevents the simultaneous firing of both M-cells<sup>22</sup>. Rapid pressure change provides a stimulus strength which saturates these inhibitory inputs and depolarizes both M-cells towards threshold. By ensuring greater inhibition of the M-cell contralateral to the sound source, the directional nature of particle motion could determine which cell fires first, and ensures the activation of one M-cell before the other. This activation inhibits the contralateral M-cell<sup>23</sup> and thus determines the direction of the escape movement<sup>24,25</sup>. This effectively preserves any directional information received from particle motion and functionally imposes it on the nondirectional pressure stimulus that initiates the escape. Thus, by discriminating between two forms of acoustic information, fish use separate components of the same stimulus to determine the onset time and direction of the behavioural response.

The presence of Weberian ossicles, a specialized structure connecting the swimbladder to the inner ear, seems to broaden the spectral range for hearing<sup>12</sup> in otophysan fishes<sup>26</sup> such as goldfish, and may also enhance the acoustic range of sound-initiated escape. Our experimental approach can be used both to study the neuroethological role of such specializations and to make comparative assessments of the relative hearing capabilities of various groups of fishes.

The M-cells are present in primitive fishes and pre-date the evolution of the swimbladder<sup>27</sup>. Thus, the successful expression of Mauthner-mediated escape could have influenced the evolution of the swimbladder for acoustic pressure transduction. Originally adaptive as a respiratory organ in hypoxic environments<sup>28,29</sup>, the swimbladder subsequently became associated with buoyancy control in species that radiated into less hypoxic waters. Although the nature of the transition from buoyancy control to hearing is unknown, we suggest that sound pressure-evoked M-cell activation and predator evasion co-evolved with, and facilitated, the addition of hearing to swimbladder function.

Received 30 April; accepted 6 August 1990.

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ACKNOWLEDGEMENTS. Supported by funding from the NIH. We thank R. DiDomenico, D. Faber, J. Hanken, S. Mizumori, A. Popper and G. Rose for helpful comments, and M. Foreman for technical assistance.

## Proliferation and differentiation of neuronal stem cells regulated by nerve growth factor

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**NERVE growth factor plays an important part in neuron–target interactions in the late embryonic and adult brain. We now report that this growth factor controls the proliferation of neuronal precursors in a defined culture system of cells derived from the early embryonic brain. Neuronal precursor cells were identified by expression of the intermediate filament protein nestin. These cells proliferate in response to nerve growth factor but only after they have been exposed to basic fibroblast growth factor. On withdrawal of nerve growth factor, the proliferative cells differentiate into neurons. Thus, in combination with other growth factors, nerve growth factor regulates the proliferation and terminal differentiation of neuroepithelial stem cells.**

Cell marking experiments in vertebrates<sup>1–7</sup> and genetic studies in *Drosophila*<sup>8,9</sup> have shown that extrinsic signals regulate the transition of a multipotential precursor to post-mitotic neurons of different types (for review see ref. 10). To identify these signals, embryonic rat striatum primordia were dissected at the time when neurogenesis begins<sup>11</sup> and cultured in serum-free medium. Immediately after plating, more than 95% of rat striatal cells dissociated from embryonic day 13.5–14.5 expressed nestin, an intermediate filament protein specifically found in neuroepithelial stem cells<sup>12,13</sup>. After 2 days *in vitro* most cells were still nestin positive (nestin<sup>+</sup>) (Fig. 1a, dashed line) whereas the 200K subunit (relative molecular mass 200,000) of neurofilament was detectable in a smaller number of cells (Fig. 1a, solid line). After 9 days *in vitro*, the cells differentiated into neurons (Fig. 1a). These results confirm previously published data that show that cells dissociated from embryonic striatum can differentiate into neurons<sup>14</sup>.

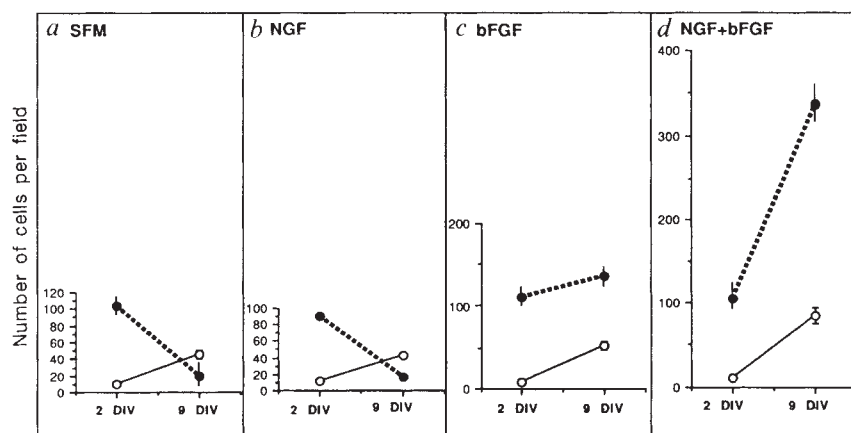
The receptor for nerve growth factor (NGF-R) has been localized in many cells throughout the embryonic striatum from embryonic day 14 onward<sup>15</sup>. When, however, NGF was added to dissociated cultures of embryonic striatum, there was no effect on the proliferation, survival or differentiation of this population of nestin<sup>+</sup> cells (Fig. 1b). Recent experiments suggest that multiple interactions between growth factors occur through the reciprocal regulation of their receptors<sup>16</sup>. We therefore determined whether striatal precursor cells became responsive to NGF after treatment with basic fibroblast growth factor (bFGF).

Recombinant bFGF promoted the proliferation and/or survival of nestin<sup>+</sup> cells during 9 days *in vitro* (Fig. 1c). The increase in the number of nestin<sup>+</sup> cells in the presence of bFGF correlated with the formation of small colonies of nestin<sup>+</sup> cells, which became visible after 3–4 days. After 9 days in culture these nestin<sup>+</sup> colonies contained no more than 5–10 cells (Fig. 2a, b). When NGF was added together with bFGF, 17 times more nestin<sup>+</sup> cells were found (85% of the cells were nestin<sup>+</sup>) after 9 days *in vitro* compared with the serum-free medium control experiment and 2.5-times more when compared to bFGF-treated cultures (Fig. 1d). With NGF and bFGF, larger colonies of between 25 and 60 cells were seen in the cultures, compared with bFGF alone. All the cells in these colonies were strongly nestin<sup>+</sup> (Fig. 2c, d).

To determine whether the colonies were the product of a single proliferating cell<sup>17</sup>, cells were infected with a dilute

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FIG. 1 Effect of NGF and bFGF on the number of nestin<sup>+</sup> and neurofilament<sup>+</sup> cells. The number of nestin<sup>+</sup> (---) and neurofilament<sup>+</sup> (—) cells after 2 and 9 days *in vitro* (2 or 9 DIV) is shown for different culture conditions. Rat striata from embryonic days 13.5–14.5 were dissected and transferred into Petri dishes containing Ca<sup>2+</sup>, Mg<sup>2+</sup>-free Hanks' balanced salt solution (HBSS) pH 7.2. After removing the meninges the tissue pieces were rinsed in HBSS and pelleted for 5 min, 1,000 r.p.m., 4 °C. A single cell suspension was obtained by gentle mechanical dissociation without the use of trypsin. Cells were counted in a haemocytometer and viability determined by exclusion of trypan blue. Cells (45,000 cm<sup>-2</sup>) were then plated in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum (FCS) into polyornithine-coated (15 µg ml<sup>-1</sup>) glass coverslips in 24-well tissue culture plates and cultured at 33 °C in 5% CO<sub>2</sub>. 15 h later the cultures were rinsed twice with HBSS and serum-free medium (SFM) (composition 1:1 F12:DMEM including: 5 µg ml<sup>-1</sup> insulin, 100 µg ml<sup>-1</sup> transferrin, 20 nM progesterone, 30 nM selenium salt, 60 µM putrescine, 2 mM glutamine, 0.11 mg ml<sup>-1</sup> sodium pyruvate, 3.7 mg ml<sup>-1</sup> sodium bicarbonate, 4.3 mg ml<sup>-1</sup> HEPES buffer) was added. Cells were cultured in SFM supplemented with growth factors where indicated; NGF (150 ng ml<sup>-1</sup>; Collaborative Research) was replaced every 2 days, recombinant bFGF (5 ng ml<sup>-1</sup>, Amgen) was applied once at day 1. After 2 and 9 days of culture, the total number of cells was measured by counting 10 fields (0.25 mm<sup>2</sup> each) across the well. Three separate wells were counted for each treatment. After counting, the cultures were fixed in 4% paraformaldehyde in phosphate buffer and immunostained. Following per-



meabilization with 0.01% Triton, cells were incubated with a 1:1,000 dilution of a rabbit antiserum against nestin (M. Marvin and R. McK.) or with a 1:160 dilution of a mouse monoclonal antibody against the 200K subunit of the neurofilament protein (ICN). After 1 h at room temperature the cells were rinsed in PBS and then incubated for 1 h respectively with a fluorescein-conjugated goat anti-rabbit (1:100, Cappel) or a rhodamine-conjugated goat anti-mouse (1:100, Cappel) secondary antibody. The percentage of immunofluorescent nestin<sup>+</sup> (---) and neurofilament<sup>+</sup> (—) cells was determined on >400 cells. The data represent the average absolute number  $\pm$  s.e.m. of nestin<sup>+</sup> and neurofilament<sup>+</sup> cells counted as described above. The results shown represent one of five independent experiments that reproduced these effects.

suspension of retrovirus expressing the *Escherichia coli lacZ*. Figure 3 shows one isolated colony containing >20 lacZ-positive cells which was expanded for 5 days *in vitro* in the presence of NGF and bFGF following infection after one day *in vitro*. These data show that the formation of colonies was a result of clonal expansion of nestin<sup>+</sup> precursor cells.

In Fig. 4 we show that NGF alone can support precursor cell proliferation after a brief exposure to bFGF. The dose-response curve shows that the number of cells was a linear function of NGF concentration in the presence (plain line) or absence (dashed line) of bFGF. The proliferation of striatal cells in response to NGF concentrations of hundreds of nanograms per millilitre may be explained by binding to the low-affinity NGF receptor. The specificity of the proliferative effect of NGF was confirmed by showing that incubating the cells with a mono-

clonal antibody to NGF completely blocked the increase in cell number. After two days pretreatment with bFGF (5 ng ml<sup>-1</sup>), the cultures were rinsed and switched either to serum-free medium or to serum-free medium containing NGF (300 ng ml<sup>-1</sup>) for three days. At this time, a 10-fold molar excess of an anti-NGF antibody (Boehringer) was applied to a set of cultures containing NGF. After six additional days in the presence of the antibody the total number of cells did not increase ( $166 \pm 12$ ) when compared with the cultures not treated with the antibody ( $439 \pm 14$ ). The smaller number of cells in the presence of anti-NGF than in serum-free medium ( $221 \pm 16$ ) might be explained by the secretion of NGF by striatal cells in culture. Furthermore, combination of NGF or bFGF with other growth factors did not produce any effect on cell number (data not shown). The observed effect of NGF may also be indirect, acting through a

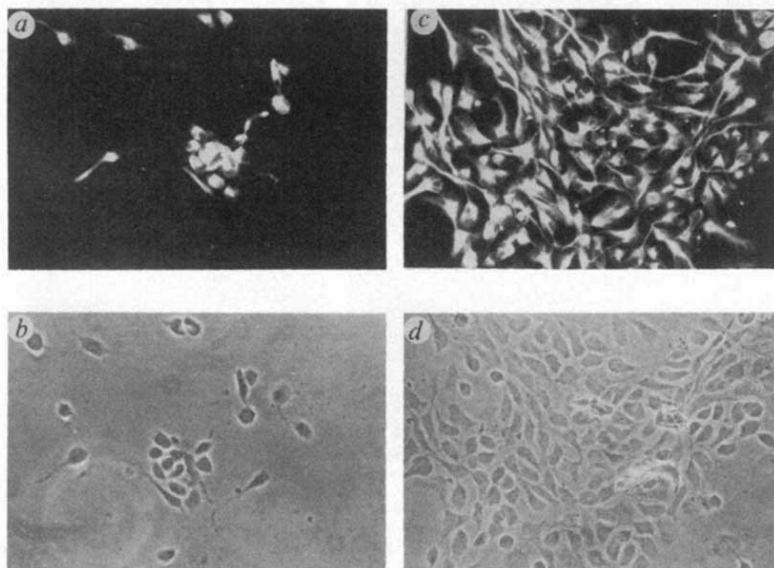


FIG. 2 Colonies of nestin<sup>+</sup> cells formed as a consequence of proliferation in the presence of bFGF and NGF. In the presence of bFGF, NGF stimulated the proliferation of colonies of striatal nestin<sup>+</sup> cells. After 9 days *in vitro* in bFGF alone (a, b) or bFGF and NGF (c, d), cells were immunostained with the anti-nestin antibody as described in the legend to Fig. 1. a, c, Fluorescent and b, d, phase-contrast pictures.



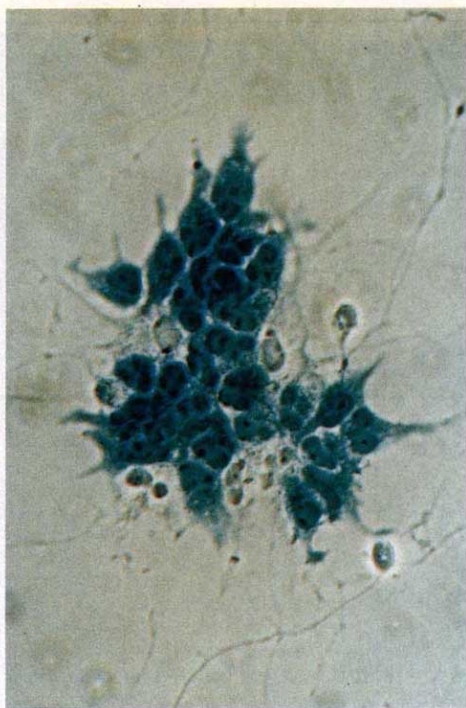


FIG. 3 Clonal expansion forms the nestin<sup>+</sup> colonies. After 1 day *in vitro*, striatal cultures were infected with a diluted suspension of a retrovirus carrying the *lacZ* gene. The supernatant was previously titrated to have 1–3 infected cells in the dish. After 5 days in the presence of NGF (150 ng ml<sup>-1</sup>) and bFGF (5 ng ml<sup>-1</sup>), cells were fixed in 0.1% glutaraldehyde, permeabilized with 0.1% Triton X-100 and reacted with 1 mg ml<sup>-1</sup> X-gal. Isolated clones of *lacZ*<sup>+</sup> cells were visible when NGF and bFGF (or NGF after a pretreatment with bFGF) were present in the culture medium. Note that the colony shown contains a clone of blue cells and a few cells which did not express  $\beta$ -galactosidase. In control cultures (no growth factors) 2–3 isolated, single, blue cells were visible in the dish. In an additional control experiment the cultures were fixed after just three days of exposure of the growth factors. In this case there were fewer cells forming the blue colonies.

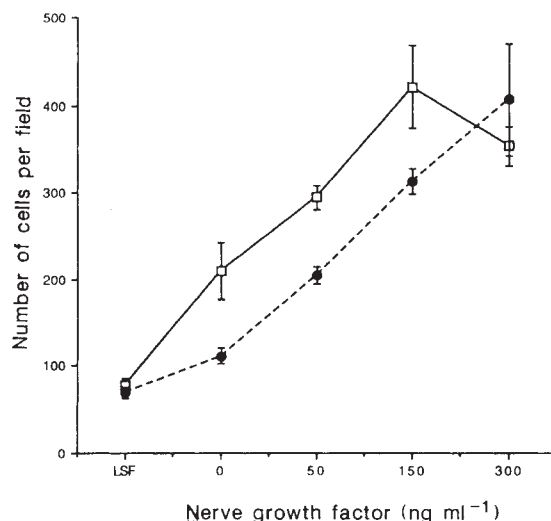


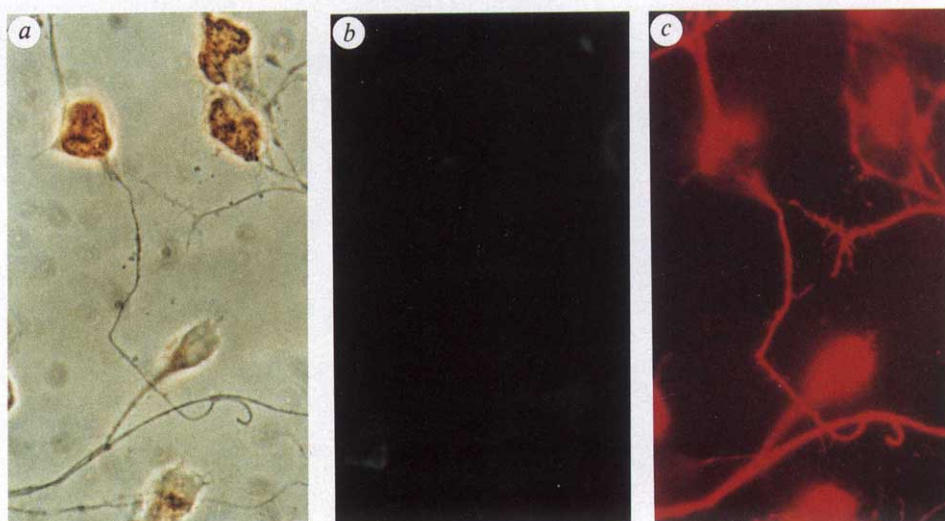
FIG. 4 Influence of NGF concentration on the total number of cells after 9 days *in vitro*. Striatal cultures were prepared as described above and cultured in the presence of various concentrations of NGF for 9 days. Cells were either cultured with NGF and bFGF (5 ng ml<sup>-1</sup>, —) or after 2 days of treatment with bFGF the medium was replaced with medium containing NGF alone (---). The total number of cells was scored as described in the legend to Fig. 1 and the results shown are the average cell number  $\pm$  s.e.m. In the figure, NGF '0 ng ml<sup>-1</sup>' represents the experimental condition in which bFGF alone is present in the medium during the whole experiment (—) or bFGF for only the two initial days and then replaced with SFM with no NGF (---). During the experiment, NGF was replaced every 48 h. These results represent one of three independent experiments.

subset of cells in the culture which then release some other proliferative factor. Despite these qualifications, the linear dose-response curve and the antibody-blocking experiment show that NGF causes proliferation of nestin<sup>+</sup> cells in a completely defined culture medium.

Actively proliferating cells can be identified by their incorporation of the nucleotide analogue 5-bromodeoxyuridine (BrdU). BrdU uptake shows that even after 9 days *in vitro* in the presence of bFGF and NGF many cells were still proliferating. When the growth factors were removed after 9 days *in vitro*, prolifer-

FIG. 5 Proliferating cells differentiated upon removal of growth factors. After 9 days *in vitro* in the presence of bFGF (5 ng ml<sup>-1</sup>) and NGF (150 ng ml<sup>-1</sup>) the cultures were shifted to fresh SFM for four additional days. Actively proliferating NGF-responsive nestin<sup>+</sup> cells were labelled with 5-bromodeoxyuridine (10  $\mu$ M BrdU) for 6 h before withdrawal of growth factors. After 18 h in SFM the flat nestin<sup>+</sup> cells comprising the colonies started to round up and after 4 days a clear morphological and immunochemical differentiation was evident in the colonies. Immunocytochemistry with anti-BrdU antibody showed that 30% of the cells were labelled (data not shown), indicating that a large proportion of the cultured cells were still actively dividing after 9 days *in vitro*. In growth-factor-free medium the BrdU-labelled cells within colonies assumed small cell bodies with fine processes (a), downregulated nestin (b) and expressed neurofilament immunoreactivity (c). Four BrdU-labelled nuclei can be seen in a.

**METHODS.** After 4 days in SFM, cells were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer and processed for triple labelling with anti-BrdU, anti-nestin and anti-neurofilament antibodies. Following denaturation in 1 N HCl and neutralization in 0.1 M sodium borate, cells were incubated for 15 h with an anti-BrdU (Becton-Dickinson 1:10 in 3% goat serum, 0.1% triton in



PBS). After rinsing in PBS, a horseradish peroxidase conjugated goat antimouse secondary antibody was applied for 1 h (Biorad 1:30) and visualized with diaminobenzidine (DAB) histochemistry. Nestin and neurofilament staining were performed after the diaminobenzidine reaction following the protocol described in the legend to Fig. 1.

ation ceased and the cells went through a clear morphological change, nestin expression was greatly reduced and fine neurofilament positive processes were seen (Fig. 5). These results show that cells which proliferated *in vitro* could still differentiate into neurons.

NGF promotes survival and neurite extension of neurons in the peripheral nervous system of vertebrates<sup>18</sup>, but also affects differentiated neurons in the central nervous system *in vitro*<sup>19,20</sup>

and *in vivo*<sup>21–24</sup>. Our experiments suggest a developmental role for NGF consistent with the early presence of NGF-R protein<sup>15</sup> and NGF messenger RNA<sup>25</sup> in the striatum. The recent discovery of two closely related growth factors, brain-derived neurotrophic factor and neurotrophin-3 (BDNF and NT-3) suggests that NGF and other members of the NGF family might promote both the proliferation of neuronal precursors and the survival/differentiation of neurons derived from these precursors<sup>26–28</sup>. □

Received 25 June; accepted 3 September 1990.

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ACKNOWLEDGEMENTS. Supported by the National Institutes of Health and the Pew Charitable Trust. R.M. is a scholar of the Rita Allen Foundation.

## Potassium conductances in hippocampal neurons blocked by excitatory amino-acid transmitters

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**EXCITATORY amino acids mediate fast synaptic transmission in the central nervous system through the activation of at least three distinct ionotropic receptors: *N*-methyl-D-aspartate (NMDA), the  $\alpha$ -amino-3-hydroxy-5-methyl-isoxasole-4-propionate (AMPA)/quisqualate (QUIS) and the kainate subtypes (for reviews, see refs 1, 2). They also activate the additional QUIS 'metabotropic' receptor (sensitive to *trans*-1-amino-cyclopentyl-1,3-dicarboxylate, ACPD) linked to inositol phospholipid metabolism<sup>3–5</sup>. We have used hippocampal slice cultures to study the electrophysiological consequences of the metabotropic response. We find that activation of an ACPD-sensitive QUIS receptor produces a 'slow' excitation of CA3 pyramidal cells, resulting from depression of a  $\text{Ca}^{2+}$ -dependent  $\text{K}^+$  current and a voltage-gated  $\text{K}^+$  current. Combined voltage-clamp and microfluorometric recordings show that, although these receptors can trigger an increase in intracellular  $\text{Ca}^{2+}$  concentration<sup>6–8</sup>, suppression of  $\text{K}^+$  currents is independent of changes in intracellular  $\text{Ca}^{2+}$ . These effects closely resemble those induced by activating muscarinic acetylcholine receptors in the same neurons and suggest that excitatory amino acids not only act as fast ionotropic transmitters but also as slow neuromodulatory transmitters.**

In hippocampal neurons, activation of QUIS 'metabotropic' receptors induces  $\text{Ca}^{2+}$ -release from intracellular stores<sup>6–8</sup> and reduces voltage-dependent  $\text{Ca}^{2+}$  currents<sup>9</sup>. All the experiments described here (with the exception of those in Fig. 2A) were carried out in the presence of 2–3 mM kynurenate. Similar results were obtained using a combination of D-2-amino-5-amino-5-phosphonovalerate (D-APV, 40  $\mu\text{M}$ ) and 6-cyano-7-nitro-quin-

oxaline-2,3-dione (CNQX, 20–100  $\mu\text{M}$ ). Under these conditions, where the excitatory effects of NMDA, kainate and AMPA were blocked, glutamate still depolarizes CA3 neurons (Fig. 1), markedly increases the number of spikes elicited by a prolonged depolarizing current pulse and blocks the slow afterhyperpolarization (AHP) following spike discharge<sup>10–12</sup>. These effects are reversible, concentration-dependent, do not desensitize with repeated applications and occur in the same concentration range as the ionotropic responses.

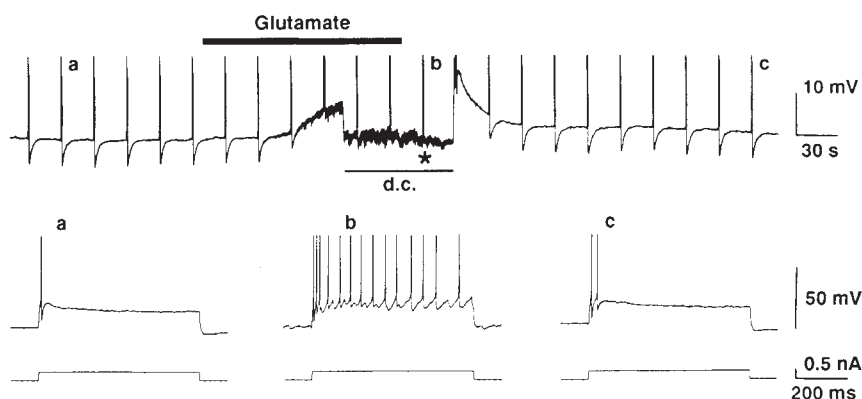
In our experiments, carried out in voltage-clamp and in the presence of tetrodotoxin (TTX, 1  $\mu\text{M}$ ), the current underlying the AHP was generated by depolarizing voltage steps (50 ms) and seen as a slow outward tail current following the depolarization (Figs 2, 3). To investigate whether the excitation induced by glutamate (Fig. 1) was due to activation of QUIS 'metabotropic' receptors<sup>4–8,13</sup>, we compared the effect of QUIS (1  $\mu\text{M}$ ) with that of AMPA (1–2  $\mu\text{M}$ )  $\pm$  2 mM kynurenate (Fig. 2A). In control solution (TTX), both AMPA and QUIS induce an inward current, but only QUIS blocks the AHP-current. Kynurenate completely abolishes the inward current produced by AMPA, whereas the effects of QUIS persist ( $n = 5$ ). This effect of low concentrations of QUIS (0.02–1  $\mu\text{M}$ ) is only weakly affected by ionotropic receptor antagonists, whereas higher concentrations of QUIS ( $> 1 \mu\text{M}$ ) produce a kynurenate-sensitive component of the inward current. Application of the putative endogenous excitatory amino-acid transmitters (0.25–1 mM) glutamate, aspartate, homocysteate<sup>14</sup> and cysteine<sup>15</sup> ( $n = 4–33$ ) reversibly induce an inward current and reduce or abolish the outward tail current. The results suggest that these novel effects of amino acids<sup>16,17</sup>, clearly different from those resulting from activation of kainate receptors<sup>18</sup>, were mediated through the activation of non-AMPA/QUIS receptors. These are most probably metabotropic receptors; indeed other metabotropic agonists<sup>19</sup> such as ibotenate (10  $\mu\text{M}$ ,  $n = 3$ ) or ACPD (10  $\mu\text{M}$ ,  $n = 4$ ) mimic the effects of QUIS.

We found that at least two distinct  $\text{K}^+$  conductances are reduced by QUIS. There is considerable evidence that the AHP current blocked by QUIS corresponds to the current which had been termed  $I_{\text{AHP}}$  (ref. 20). First, its reversal potential shifts by 22 mV, from  $-100 \pm 4.5$  to  $-78 \pm 4.0$  mV ( $\pm$  s.e.m.) ( $n = 5$ ) when extracellular  $\text{K}^+$  was increased from 2.7 to 8.1 mM, a value which is close to the 27-mV shift expected from the Nernst



FIG. 1 Effects of excitatory amino acids on spike afterhyperpolarization and accommodation of a CA3 pyramidal cell in hippocampal slice culture. Continuous perfusion with a solution containing kynurenate (2 mM). *a*, Top trace, current-clamp recording of a CA3 pyramidal cell; resting membrane potential  $-51$  mV. Depolarizing current pulses were injected at  $0.05$  Hz and selected responses are illustrated in bottom traces (action potentials are truncated). Glutamate, bath-applied at  $500$   $\mu$ M, reversibly depolarized the neuron, reduced cell accommodation of firing, abolished the slow AHP (asterisk) following the depolarization pulse and increased the rate of spontaneous synaptic potentials. *b*, The number of spikes evoked by a depolarizing pulse increased during application of glutamate, even though the membrane potential was manually clamped close to its resting value (d.c. for negative direct current injection).

**METHODS.** Hippocampal organotypic cultures were prepared from 5- to 6-day-old Wistar rats as described previously<sup>30</sup>. After 2-4 weeks *in vitro*, the cultures were placed in a recording chamber, mounted on the stage of an inverted microscope and perfused with an external solution ( $32$   $^{\circ}$ C) containing (mM):  $\text{Na}^+$ , 148.9;  $\text{K}^+$ , 2.7;  $\text{Cl}^-$ , 148.2;  $\text{Ca}^{2+}$ , 3.8;  $\text{Mg}^{2+}$ , 0.5;  $\text{HCO}_3^-$ ,



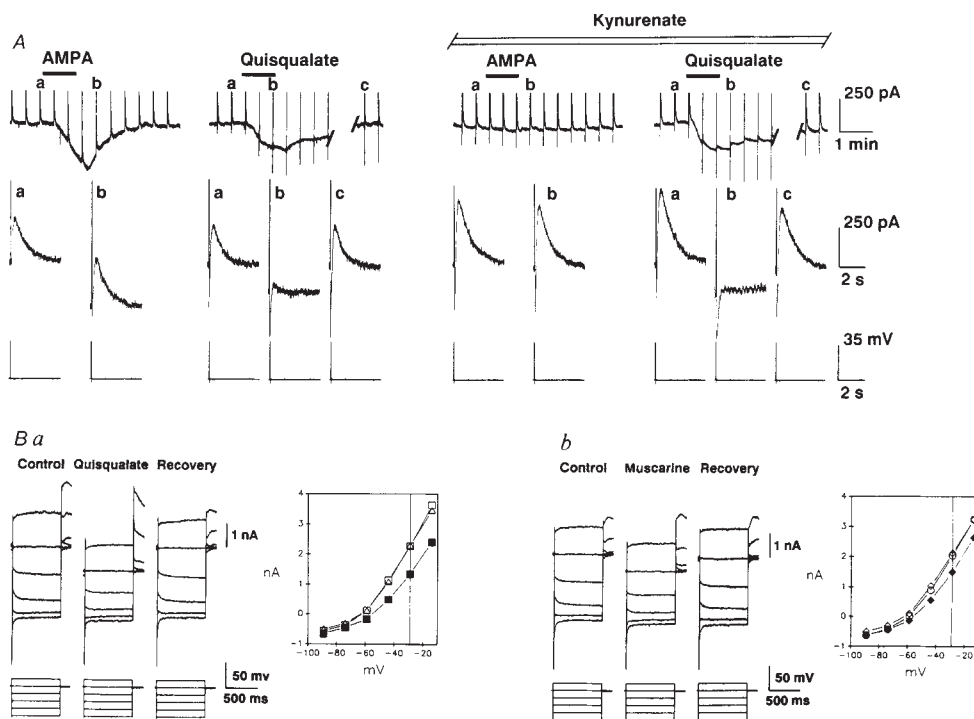
11.6;  $\text{H}_2\text{PO}_4^-$ , 0.4; D-glucose, 5.6. Intracellular recordings were performed with micropipettes containing 2 M potassium methyl sulphate, unless specified. Current- and voltage-clamp recordings were carried out using an Axoclamp 2 amplifier (Axon Instruments). All drugs were added to the perfusion solution. Results reported here are based on recordings from 75 CA3 pyramidal cells.

equation. Second, it persists in the presence of extracellular tetraethylammonium (TEA, 10 mM),  $\text{Cs}^+$  (2 mM) and 4-aminopyridine (200  $\mu$ M–1 mM). Third, it is blocked by addition of  $\text{Cd}^{2+}$  (50–100  $\mu$ M) or by removal of extracellular  $\text{Ca}^{2+}$  ( $\text{Ca}^{2+}$ , 0 mM;  $\text{Mg}^{2+}$ , 10 mM) from the perfusion solution. Finally, it is sensitive to isoproterenol (2.5  $\mu$ M,  $n=4$ ), histamine (10  $\mu$ M,  $n=3$ ) and muscarine (0.5–1  $\mu$ M,  $n=6$ ), agonists known to reduce  $I_{\text{AHP}}$  (refs 21–23). The blockade of the AHP current

could result from the depression of  $\text{Ca}^{2+}$  currents<sup>9</sup>. But in our preparation,  $I_{\text{AHP}}$  was blocked at concentrations of QUIS that had no effect on the depolarization-induced change in cytosolic free- $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_i$ ) (see below) and on  $\text{Ca}^{2+}$  action potentials recorded in current clamp (TEA concentration, 5–10 mM;  $n=4$ ).

The inward current induced by QUIS is associated with a membrane conductance decrease and results from the blockade

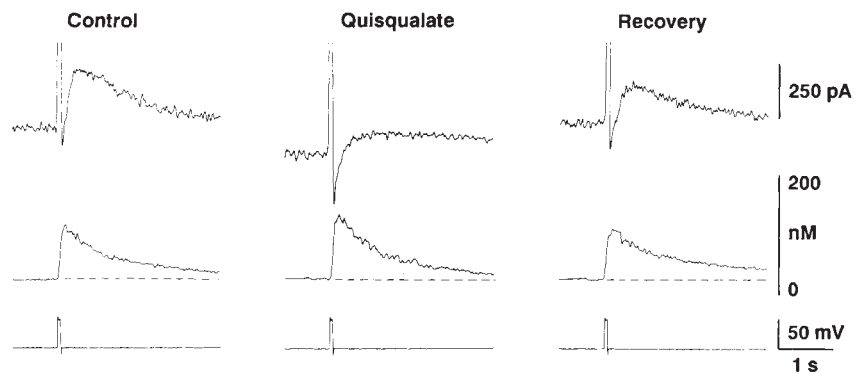
FIG. 2 *A*, Differential effects of quisqualate and AMPA. A CA3 pyramidal cell voltage clamped at  $-60$  mV and continuously perfused with a solution containing TTX (1  $\mu$ M). Quisqualate (0.5  $\mu$ M) but not AMPA (1  $\mu$ M) blocked  $I_{\text{AHP}}$ . Short depolarizing voltage jumps (50 ms), applied at 0.04 Hz, induced outward tail currents ( $I_{\text{AHP}}$ ). Bottom traces, examples of tail currents selected from the top trace. Left, application of AMPA (1  $\mu$ M) induced an inward current without affecting  $I_{\text{AHP}}$  while quisqualate (0.5  $\mu$ M) induced an inward current associated with a blockade of  $I_{\text{AHP}}$ . Right, addition of kynurenate (2 mM) to the bathing solution abolished the response to AMPA but not to quisqualate. *B*, Effect of quisqualate on the voltage-dependent  $\text{K}^+$  current  $I_{\text{M}}$ . Another CA3 neuron was voltage-clamped at  $-29$  mV, perfused with a solution containing TTX (1  $\mu$ M), kynurenate (2 mM), cadmium (50  $\mu$ M) and recorded with a KCl-filled micropipette. Exposure to cadmium increased the membrane conductance and generated an outward current that stabilized after 5 min. *Ba*, left, hyperpolarizing steps reveal time- and voltage-dependent current relaxations corresponding to a deactivation of  $I_{\text{M}}$ . The direction of these relaxations reverse at a value close to the expected  $\text{K}^+$  equilibrium potential ( $-85$  mV;  $[\text{K}^+]_{\text{ext}}$  5.4 mM). Quisqualate (1  $\mu$ M) reversibly induces an inward current, reduces the membrane conductance and decreases the amplitude of the current relaxations. Right, current-voltage relationships before ( $\square$ ), during ( $\blacksquare$ ) and after ( $\triangle$ ) the



application of quisqualate. Current values obtained 50 ms before the end of the voltage step. *Bb*, Muscarine (1  $\mu$ M) acts similarly to quisqualate. Right, current-voltage relationships before ( $\diamond$ ), during ( $\blacklozenge$ ) and after ( $\circ$ ) application of muscarine.

FIG. 3 Quisqualate blockade of  $K^+$  currents is independent of changes in cytosolic free- $Ca^{2+}$  ( $[Ca^{2+}]_i$ ). Combined voltage-clamp and microfluorometric (Fura-2) recordings of a CA3 pyramidal cell perfused with a solution containing TTX ( $1 \mu M$ ), kynurenatate ( $2 mM$ ) and voltage-clamped at about  $-55 mV$ . Voltage jumps of 50 ms duration to about  $-5 mV$  induce slow outward tail currents associated with a transient increase in  $[Ca^{2+}]_i$ . Clamp current (upper traces),  $[Ca^{2+}]_i$  (middle traces), and voltage (lower traces). Application of quisqualate ( $1 \mu M$ ) results in a reversible depression of the slow tail currents though  $[Ca^{2+}]_i$  transients are not depressed (they probably increase because of the reduced  $K^+$  conductances) and resting  $[Ca^{2+}]_i$  remains unaffected.

**METHODS.** Neurons were impaled with thin-walled microelectrodes, the tip of which contained Fura-2 ( $1 mM$ ) and  $300 mM$   $KMeSO_4$  and backfilled with  $2 M$   $KMeSO_4$ . After intracellular injection of the dye ( $-0.3$  to  $-0.6 nA$ , constant current for 20–30 min) epifluorescence was excited either at 366 or 405 nm using a mercury arc lamp. To measure Fura-2 fluorescence



intensity, an image of the cell body was projected through a 470-nm barrier filter onto a photodiode. Calculation of  $[Ca^{2+}]_i$  from Fura-2 fluorescence intensities was based on a modified ratio method<sup>27</sup> using calibration constants evaluated from calibration solutions (pH 7.4) containing  $131 mM$   $K^+$ ,  $10 mM$   $Na^+$ ,  $1 mM$   $Mg^{2+}$ ,  $123.5 mM$   $Cl^-$ ,  $5 mM$  HEPES,  $10 \mu M$  fura-2,  $5 mM$  of the  $Ca^{2+}$  buffer BAPTA and varying concentrations of free  $Ca^{2+}$ .

of a second, tonically activated  $K^+$  current. The effect, present only at potentials more positive than about  $-70 mV$ , is similar when KCl-filled micropipettes are used. This voltage-dependent current is carried, at least in part, by  $K^+$  ions as the current-voltage relationship changes with the extracellular  $K^+$  concentration (not shown).

A large fraction of the inward current does not seem to be  $Ca^{2+}$ -dependent. Indeed, the inward current persists in the presence of  $Cd^{2+}$  ( $50$ – $100 \mu M$ ,  $n = 7$ ), in  $Ca^{2+}$ -free ( $Ca^{2+}$ ,  $0 mM$ ;  $Mg^{2+}$ ,  $10 mM$ ,  $n = 3$ ) solutions and when recordings were carried out with BAPTA filled microelectrodes ( $n = 4$ ), conditions which abolish  $I_{AHP}$ . In the presence of  $50 \mu M$   $Cd^{2+}$  (Fig. 2B) (holding potential of  $-29 mV$ ), hyperpolarizing voltage steps produced time- and voltage-dependent inward relaxations that reverse between  $-74$  and  $-89 mV$ , close to the estimated  $K^+$  equilibrium potential of  $-85 mV$ , and resemble the deactivation of  $I_M$  (refs 24–26). QUIS, like muscarine, decreases membrane conductance and abolishes time- and voltage-dependent current relaxations. The QUIS inward current is also blocked by  $1 mM$  barium ( $n = 3$ ) and can be occluded by application of muscarine ( $0.5$ – $1 \mu M$ ) ( $n = 3$ ), but QUIS is insensitive to the muscarinic antagonist atropine ( $1 \mu M$ ;  $n = 6$ ). Our results suggest that inhibition of  $I_M$  contributes to the inward current induced by QUIS.

Combining microfluorometric (Fura-2) measurements of  $[Ca^{2+}]_i$  with voltage-clamp recordings, we found that QUIS reversibly blocks  $I_{AHP}$  without raising the resting  $[Ca^{2+}]_i$  or depressing the  $Ca^{2+}$  transients caused by depolarizing pulses<sup>9</sup> (Fig. 3). Occasionally, QUIS also induces a short-lasting increase in the resting  $[Ca^{2+}]_i$ , as previously reported<sup>6–8</sup>, but the blockade of  $K^+$  currents outlasts this change in  $[Ca^{2+}]_i$ . We conclude that the blockade of  $I_{AHP}$  by QUIS, like that of muscarine<sup>27</sup>, is not mediated by a sustained increase in  $[Ca^{2+}]_i$  or a blockade of voltage-dependent  $Ca^{2+}$  channels.

Activation of 'metabotropic' QUIS receptors produces a form of 'slow' excitation closely resembling that of muscarinic receptors, that is, an inhibition of  $Ca^{2+}$ - and voltage-gated  $K^+$ -currents. Because both 'metabotropic' excitatory amino acids and muscarinic receptor activation stimulate phosphatidylinositol breakdown in hippocampal pyramidal cells, it seems likely that a common final path is involved. Inhibition of  $I_{AHP}$  could be due to activation of protein kinase C (ref. 28), whereas inhibition of  $I_M$  could result from a  $Ca^{2+}$ -independent action of inositol triphosphate<sup>29</sup>. Our experiments further suggest that excitatory amino acids, like acetylcholine, can act as fast ionotropic and slow modulatory transmitters. □

Received 6 July; accepted 4 September 1990.

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**ACKNOWLEDGEMENTS.** We thank D. A. Brown, P. Ascher, S. M. Thompson and E. Audinat for comments on the manuscript; L. Rietschin, J. Meier, E. Vollenweider and R. Schoeb for technical assistance. Supported by the Swiss National Science Foundation and the Dr Eric Slack-Gyr Foundation.



## Localization of nitric oxide synthase indicating a neural role for nitric oxide

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NITRIC oxide (NO), apparently identical to endothelium-derived relaxing factor in blood vessels<sup>1-3</sup>, is also formed by cytotoxic macrophages<sup>4,5</sup>, in adrenal gland<sup>6</sup> and in brain tissue<sup>7-9</sup>, where it mediates the stimulation by glutamate of cyclic GMP formation in the cerebellum<sup>10,11</sup>. Stimulation of intestinal<sup>12</sup> or anococcygeal<sup>13-15</sup> nerves liberates NO, and the resultant muscle relaxation is blocked by arginine derivatives that inhibit NO synthesis. It is, however, unclear whether in brain or intestine, NO released following nerve stimulation is formed in neurons, glia, fibroblasts, muscle or blood cells, all of which occur in proximity to neurons and so could account for effects of nerve stimulation on cGMP and muscle tone. We have now localized NO synthase protein immunohistochemically in the rat using antisera to the purified enzyme<sup>16</sup>. We demonstrate NO synthase in the brain to be exclusively associated with discrete neuronal populations. NO synthase is also concentrated in the neural innervation of the posterior pituitary, in autonomic nerve fibres in the retina, in cell bodies and nerve fibres in the myenteric plexus of the intestine, in adrenal medulla, and in vascular endothelial cells. These prominent neural localizations provide the first conclusive evidence for a strong association of NO with neurons.

To ensure that the antiserum interacts with catalytically active NO synthase (NOS, EC 1.14.23), we conducted immunoprecipitation experiments (data not shown). The antiserum precipitates NOS activity in cerebellar homogenates whether measured<sup>10</sup> by the conversion of arginine to citrulline or by the formation of NO with half-maximal precipitation at 10  $\mu\text{g ml}^{-1}$  antiserum IgG. In western blot analysis the antiserum interacts with a

single band of relative molecular mass 150,000 ( $M_r$  150K), the same as purified NOS<sup>16</sup>. The density of the band varies amongst various brain regions and subdivisions of pituitary and adrenal glands in close parallel with the regional distribution of NOS catalytic activity (data not shown). Antisera from two rabbits and affinity-purified NOS antibodies provide identical distributions by western blot analysis and by immunohistochemical staining (see below). Immunoreactivity is absent with pre-immune serum or with serum preabsorbed with purified NOS (data not shown).

Dramatic variations in immunoreactivity occur throughout rat brain (Fig. 1a). Staining is densest in cerebellar molecular and granule cell layers, and much reduced in the Purkinje cell layer. Similarly high immunoreactivity is observed in the olfactory bulb, especially in the granule cell layer. Other areas of abundant staining include the superior and inferior colliculi, the dentate gyrus of the hippocampus, the bed nucleus of the stria terminalis, the islands of Callejae and the horizontal limb of the diagonal band of Broca. Somewhat less staining is observed in superficial layers of the cerebral cortex.

Under high magnification immunoreactivity in the molecular layer of the cerebellum is revealed as most intense in cell bodies and horizontally extending processes of basket cells (Fig. 1b). In the granule cell layer, intense immunoreactivity is apparent in glomeruli, where terminals of mossy fibres and Golgi II cells synapse on granule cells which themselves also appear immunoreactive.

In the pituitary gland, the posterior lobe stains intensely, with negligible staining in the intermediate and anterior lobes (Fig. 2). At high magnification this staining is seen to occur in fibres and terminals. Intense staining of cell bodies in the supraoptic and paraventricular nuclei is also evident and may be the source of the projections to the immunoreactive fibres in the posterior pituitary.

NOS immunoreactivity is evident in numerous peripheral neural systems (Fig. 3). Within the myenteric plexus of the intestinal tract, both cell bodies and nerve fibres coursing in parallel with the inner circular muscle are strongly immunoreactive. In the retina, immunoreactivity is restricted to processes, most probably autonomic nerve fibres, making close contact with the choroid and the pigmented epithelium. In the adrenal

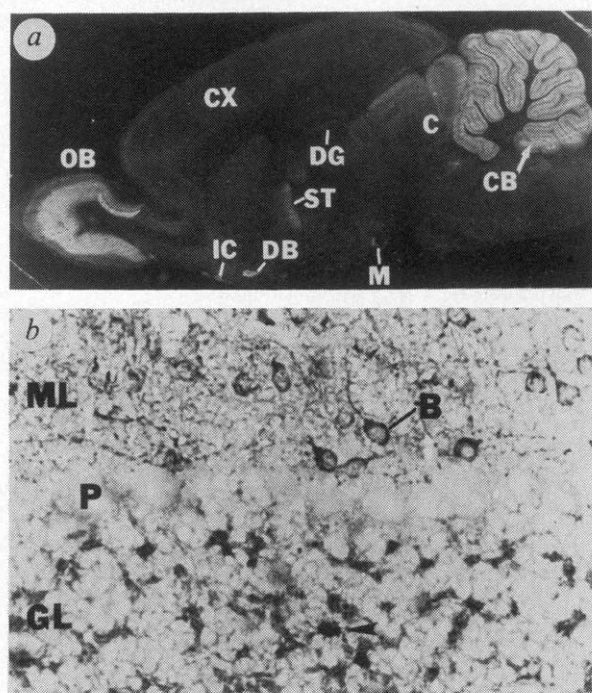


FIG. 1 Immunohistochemical localization of NOS in rat brain. *a*, Dark-field image of a sagittal brain section labelled with affinity-purified antiserum. Strongest staining (appearing white) is in the molecular and granule cell layers of the cerebellum, superior and inferior colliculi and the granule cell layer of the olfactory bulb. Lesser staining appears in the superficial layers of the cerebral cortex, the dentate gyrus of the hippocampus and the bed nucleus of the stria terminalis. Smaller regions of localized staining occur in the islands of Callejae, the diagonal band of Broca and the mammillary nuclei. CB, cerebellum; C, superior and inferior colliculi; OB, olfactory bulb; CX, cerebral cortex; DG, dentate gyrus; ST, bed nucleus of the stria terminalis; IC, islands of Callejae; DB, diagonal band of Broca; M, mammillary nuclei. *b*, High-power light-field micrograph of cerebellum ( $\times 400$ ) shows dark labelling of glomeruli (arrowhead), and granule cells, in the granule cell layer and in basket cells and their processes in the molecular layer. Purkinje cell bodies are unstained. GL, granule cell layer; ML, molecular layer; B, basket cell; P, Purkinje cell.

**METHODS.** NOS was purified by column chromatography<sup>16</sup>. Antibodies were raised in two rabbits and affinity purified with purified NOS as described previously for the inositol trisphosphate receptor<sup>17</sup>. Rats were perfused with 0.1% freshly depolymerized paraformaldehyde in PBS, tissues were dissected, transferred to plastic moulds and embedded in OCT medium (Tissue-Tek). Cryostat sections (16  $\mu\text{m}$ ) were cut on gelatin-coated glass slides and dried overnight. Affinity-purified antiserum (1:600) was applied overnight at 4  $^{\circ}\text{C}$  in PBS and 1% normal goat serum. Staining used an avidin-biotin-peroxidase system (Vector) with diaminobenzidine a chromogen. Crude serum (1:5,000) showed similar localizations to those with affinity-purified serum (data not shown). Sections incubated with pre-immune serum (1:500) or affinity-purified serum (1:600) preabsorbed with purified NOS (10  $\mu\text{g ml}^{-1}$ ) showed no staining using identical incubation and development conditions (data not shown).

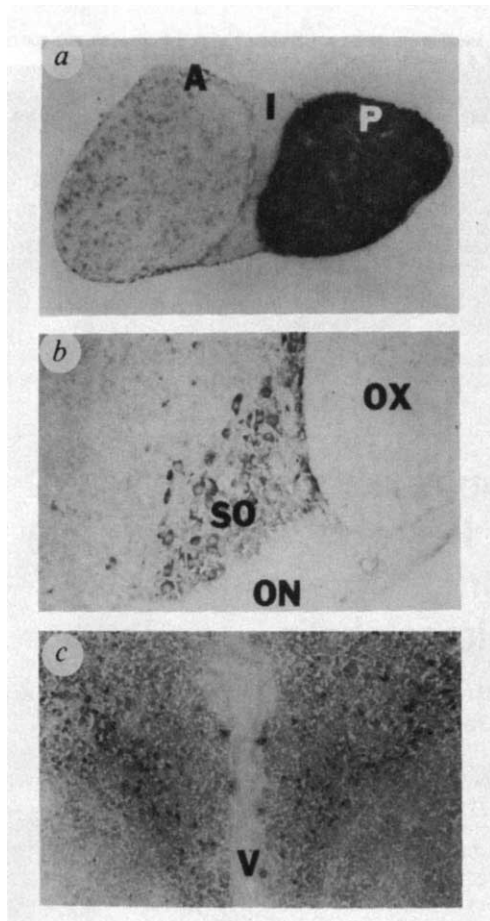


FIG. 2 Immunohistochemical localization of NOS in the pituitary, and supra-optic and paraventricular nuclei. *a*, Horizontal section of pituitary ( $\times 40$ ) showing intense labelling of the posterior lobe. P, posterior lobe; I, intermediate lobe; A, anterior lobe. *b*, Sagittal section ( $\times 400$ ) with labelling of neuronal cell bodies of the supraoptic nucleus. SO, supraoptic nucleus; OX, optic chiasm; ON, optic nerve. *c*, Coronal section ( $\times 200$ ) through the hypothalamus with strong labelling of the magnocellular neurons of the paraventricular nucleus. V, third ventricle. Sections were processed as in Fig. 1, with reaction product appearing dark.

gland, immunoreactivity is localized to the medulla with the staining pattern resembling synaptic innervation of chromaffin cells.

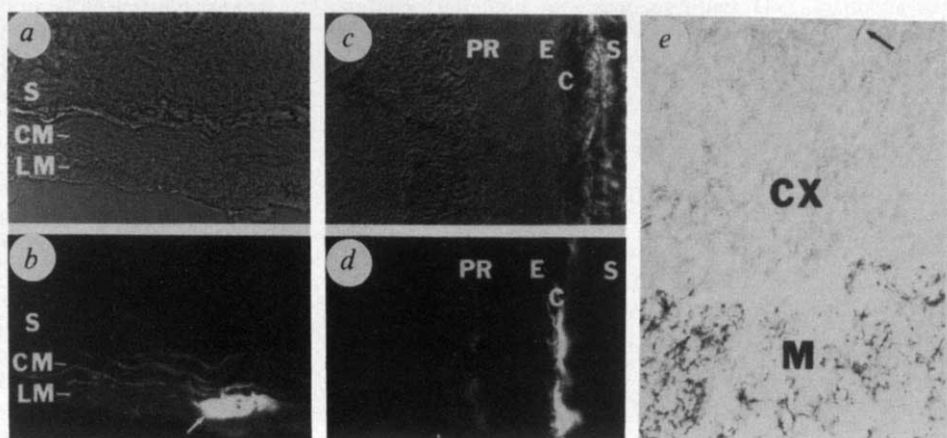
We also observe NOS immunoreactivity localized to the endothelial layers of larger blood vessels, such as the aorta, coronary arteries and blood vessels in the brain (Fig. 4), which fits with observations of NOS activity in endothelial cells<sup>18,19</sup>. Large cerebral blood vessels also display immunoreactive nerve fibres surrounding the adventitia, a pattern not evident in peripheral vessels.

The localization of NOS to neurons in the brain and periphery is dramatically selective. Amongst numerous peripheral organs examined (liver, heart, lung, spleen, kidney, thymus, testis and salivary glands) we have not observed NOS in parenchymal, fibroblast, fat, epithelial or muscle cells. Thus, the formation of NO and its functions throughout the body seem to be selectively associated with neurons and the endothelium of blood vessels. NO is known to be formed in activated macrophages by an enzyme with properties different from those of the brain-endothelial NOS<sup>20,21</sup>. Our antisera do not detect 150K NOS in western blots of activated macrophages (data not shown)<sup>4,5</sup>. Within the brain, NOS immunoreactivity is localized only to neurons and to fibres which appear to make close contact with other neurons, such as basket cells and their processes and glomerular synapses in the cerebellum. This discrete localization of NOS in certain neuronal populations in the brain sheds light on its function. In the cerebellum the proximity of basket cells to Purkinje cells is consistent with a role in enhancing cGMP formation in Purkinje cells<sup>22</sup>, which possess abundant guanylyl cyclase immunoreactivity<sup>23</sup>. NOS in cerebellar glomeruli may regulate cGMP levels in granule cells which also possess guanylyl cyclase<sup>24,25</sup> but much less abundantly than Purkinje cells.

The localizations of NOS to neuronal projections in the posterior pituitary and adrenal medulla may reflect a role for NO in regulating hormone release. This possibility is supported by findings that NO<sup>26</sup> and sodium nitroprusside<sup>27</sup>, which generates NO, stimulate catecholamine release in adrenal medullary preparations. The localization of NOS to autonomic nerves contacting smooth muscle indicates a role for NO in smooth muscle regulation beyond the actions of endothelium-derived NO on vascular muscles. Very recently, NO release was observed following electrical stimulation of intestinal<sup>12</sup>, anococcygeal<sup>13-15</sup>, and vasodilator nerves<sup>28</sup>. *N*-monomethylarginine, an inhibitor of NO synthase, prevents the associated non-adrenergic, non-cholinergic muscle relaxation. In rat duodenum, in which we directly demonstrate NO synthase in the myenteric plexus, we have reversed non-adrenergic, non-cholinergic nerve-mediated

FIG. 3 Immunohistochemical localizations of NOS in peripheral nerves. *a* and *b*, Nomarski optic and immunofluorescent images of the duodenum in cross section ( $\times 200$ ) with intense white immunofluorescence in neuronal cell bodies of myenteric plexi (arrow in *b*) and associated neuronal processes running in parallel with the inner circular muscle layer. LM, outer longitudinal muscle; CM, inner circular muscle; S, submucosa. *c* and *d*, Nomarski optic and immunofluorescent images of retina with labelling of neuronal processes in the choroid. PR, photoreceptors; E, pigmented epithelium; C, choroid; S, sclera. *e*, Immunoperoxidase staining of adrenal gland shows dark labelling of nerve fibres in the medulla with occasional fibres tracking through the cortex (arrow). CX, cortex; M, medulla.

**METHODS.** Sections were processed as in Fig. 1; *b* and *d* were labelled with avidin-linked fluorescein isothiocyanate.





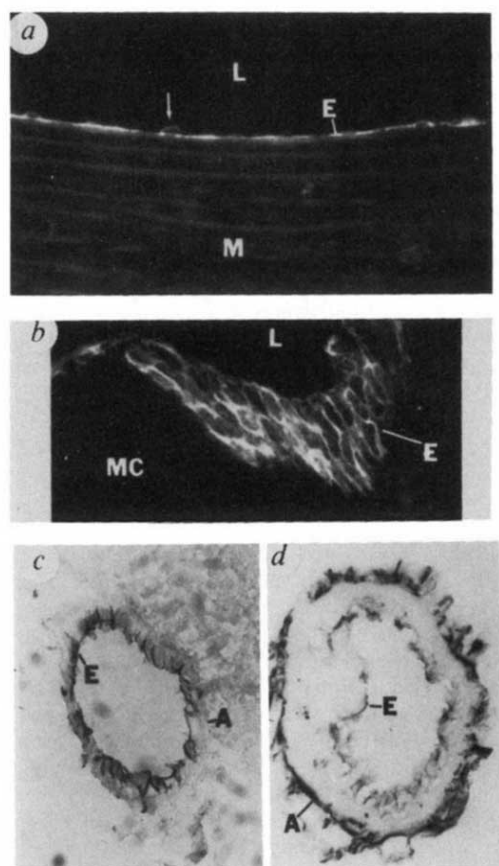


FIG. 4 Immunohistochemical localization of NOS in vasculature. *a*, Cross section of aorta ( $\times 400$ ) with strong white immunofluorescence of the endothelial cells whose nuclei can be seen bulging into the lumen (arrow). L, lumen; E, endothelial cell layer; M, tunica media. *b*, Oblique section through the coronary artery ( $\times 400$ ) with perinuclear labelling of a sheet of endothelial cells. L, lumen; E, endothelial cells; MC, myocardium. *c*, Dark immunoperoxidase staining of a distal cerebral vessel ( $\times 200$ ) shows labelling restricted to endothelial cells. *d*, Anterior communicating cerebral artery ( $\times 100$ ) shows labelling both of the endothelium as well as dense innervation about the adventitia. E, endothelium; A, adventitia. Sections were processed as Fig. 1; *a* and *b* were labelled with avidin-linked fluorescein isothiocyanate.

relaxation with benzylnitroarginine, a potent NO synthase inhibitor (J. Buzy, D.S.B., P.M.H. and S.H.S., in preparation). Thus, NO released by these nerves seems to serve as a non-adrenergic, non-cholinergic smooth muscle relaxant.

In summary, NO synthase occurs in numerous neuronal systems, implying a neural messenger role for nitric oxide, perhaps even more prominent than its functions in vascular endothelium. □

Received 5 April; accepted 11 September 1990.

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ACKNOWLEDGEMENTS. We thank A. Verma for helpful discussions and preparation of the sagittal brain sections. This work was supported by the US Public Health Service and a gift from Bristol-Myers-Squibb.

## A complex between the MHC class I homologue encoded by human cytomegalovirus and $\beta_2$ microglobulin

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HUMAN cytomegalovirus (HCMV) is a ubiquitous pathogen that persists in the host and can cause severe disease in the immunocompromised individual or in the fetus. Analysis of the nucleotide sequence of the virus genome has revealed the presence of an open reading frame whose predicted translation product has homology with the heavy chain of the major histocompatibility complex (MHC) class I molecule of higher eukaryotes<sup>1</sup>, and the observed sequence homology was given additional significance by the independent observation<sup>2</sup> that HCMV virions can bind  $\beta_2$  microglobulin ( $\beta_2m$ ), the light chain of the MHC class I molecule. We expressed both the HCMV class I homologue and the human  $\beta_2m$  gene in recombinant vaccinia viruses. We show that the coexpressed gene products associate, that the transport of  $\beta_2m$  to the cell surface is dependent on coexpression of the class I homologue and that the viral gene product is therefore functionally related to its cellular counterpart. We observe also that, in HCMV-infected cells, no synthesis of mature cellular class I molecules occurs, while messenger RNA levels remain unaltered, and we speculate that one function of the viral homologue may be to sequester  $\beta_2m$ , thus preventing the maturation of cellular class I molecules and rendering the infected cell unrecognizable by cytotoxic T cells.

To determine whether the HCMV class I heavy chain homologue (the product of the HCMV UL18 gene<sup>3</sup>) could interact with  $\beta_2m$ , recombinant vaccinia viruses expressing each of these proteins were constructed (vUL18 and v $\beta_2m$ ). As vaccinia virus 'shuts down' host cell protein synthesis, infection with either recombinant alone did not result in interaction of the expressed protein with cellular gene products. But when cells were coinfecting with both recombinant viruses, the UL18 gene product of relative molecular mass 67,000 (67K) was coprecipitated with  $\beta_2m$  by a monoclonal antibody to  $\beta_2m$  (Fig. 1). In cells infected with v $\beta_2m$  alone the recombinant product was detectable in the cytoplasm but was barely detectable at the cell surface (Fig. 2A), consistent with the finding that surface expression of  $\beta_2m$  requires class I heavy chain synthesis<sup>4</sup>. When

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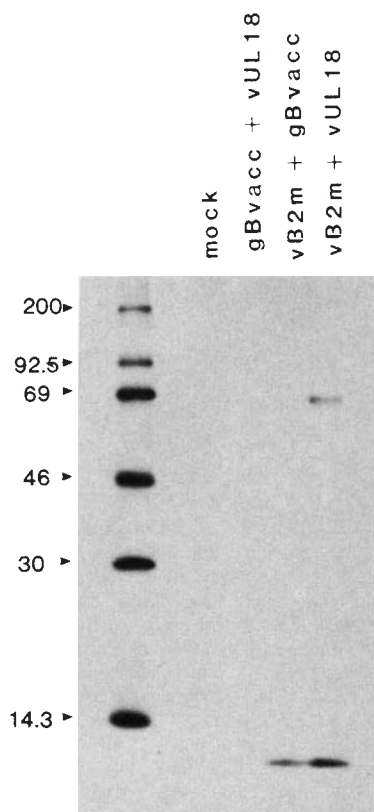


FIG. 1 Coprecipitation of  $\beta$ 2m and the HCMV UL18 gene product from BHK cells coinfecting with recombinant vaccinia viruses v $\beta$ 2m and vUL18. Lysates of BHK cells infected with gBvacc and vUL18, v $\beta$ 2m and gBvacc, v $\beta$ 2m and vUL18, and from mock-infected cells, were immunoprecipitated with monoclonal antibody BBM-1. Relative molecular mass markers (in K) are shown to the left.

**METHODS.** A 1,370-base pair (bp) *Bam*HI-*Eco*RI fragment of the *Hind*III O fragment of HCMV strain AD169 DNA, containing the entire UL18 open reading frame, was ligated into pUC18. This open reading frame was originally designated H301 (ref. 1), but has recently been renamed<sup>3</sup>. To remove an additional ATG lying 28 nucleotides upstream of the coding sequence, *Bal*31 digestion was carried out on this construct after digestion with *Bam*HI. *Bam*HI-*Eco*RI fragments were end-repaired and ligated into pUC18 *Sma*I-*Eco*RI. Resultant clones were sequenced and a plasmid, pSBH301, containing 26 nucleotides 5' of the initiating ATG of the UL18 ORF was selected. The UL18 ORF was isolated by *Bam*HI-*Eco*RI digestion of this construct and ligated into the vaccinia insertion vector pRK19 (R. K. Kent and G.S., manuscript in preparation), such that the UL18 coding sequence lies downstream of a promoter for a vaccinia virus late gene (the *4b* gene)<sup>14</sup>. A clone containing a human  $\beta$ 2m full-length complementary DNA was a gift from K. Parker and D. Wiley, and a 462-bp *Eco*RI fragment was isolated by partial endonuclease digestion and ligated into the *Eco*RI site of vaccinia insertion vector pRK19, to give pRK $\beta$ 2m. Recombinant thymidine kinase-negative vaccinia viruses, vUL18 and v $\beta$ 2m, were produced from p103 and pRK $\beta$ 2m, respectively, using established transfection and selection techniques<sup>15</sup>. A 67K polypeptide was detected by SDS PAGE and autoradiography of [<sup>35</sup>S]methionine-labelled lysates of BHK cells infected with vUL18 (data not shown), suggesting that when expressed in vaccinia the UL18 product is glycosylated, as the predicted primary translation product is ~41.7K. Monolayers of BHK cells were infected with vaccinia recombinants gBvacc and vUL18, v $\beta$ 2m and gBvacc, v $\beta$ 2m and vUL18, or were mock-infected, and labelled with [<sup>35</sup>S]-methionine from 3 to 8 h after infection (gBvacc is a vaccinia recombinant that expresses glycoprotein B of HCMV ref. 16). For double infections, each virus was used at a multiplicity of infection of 15. Infected cells were lysed in RIPA buffer (50 mM Tris-HCl, pH 7.2, 150 mM NaCl, 1% sodium deoxycholate, 0.1% SDS, 1% Triton X100) containing 2 mM PMSF and 5  $\mu$ g ml<sup>-1</sup> DNase, and lysates were incubated with the monoclonal antibody BBM1 (ref. 17). Immune complexes were collected with protein A-Sepharose, eluted in gel sample buffer containing 5% SDS and 5% 2-mercaptoethanol, and electrophoresed on 15% SDS-polyacrylamide gels. Gels were fixed, treated with Amplify (Amersham), dried and exposed to X-ray film.

cells were coinfecting with vUL18 and v $\beta$ 2m, cell-surface expression of  $\beta$ 2m increased roughly 10-fold (Fig. 2). The HCMV class I homologue therefore has functional characteristics in common with its cellular counterpart in that it binds to  $\beta$ 2m and facilitates cell surface expression of this molecule.

The resolution of the crystal structure of HLA-A2 (ref. 5) revealed that residues in the  $\alpha$ 1,  $\alpha$ 2 and  $\alpha$ 3 domains of the molecule interact with  $\beta$ 2m. Of these residues only about 50% of those in the  $\alpha$ 1 and  $\alpha$ 2 domains, and even less in the  $\alpha$ 3 domain, are conserved in the HCMV homologue. Nevertheless, this protein forms a heterodimer with  $\beta$ 2m. The question remains, however, as to whether this phenomenon is related to the observation that HCMV virions are found coated with  $\beta$ 2m. In fact the data are not entirely consistent with this view; whereas we observe association of endogenous  $\beta$ 2m with the class I homologue, Grundy and colleagues<sup>2,6</sup> found that virions associate with exogenous  $\beta$ 2m. Furthermore, Grundy *et al.*<sup>7</sup> have adduced evidence that  $\beta$ 2m-coated virus uses the cellular class I molecule as a receptor, and it is clear that  $\beta$ 2m could not interact simultaneously with a class I molecule on the cell surface and with a class I homologue on the virus surface. Finally,

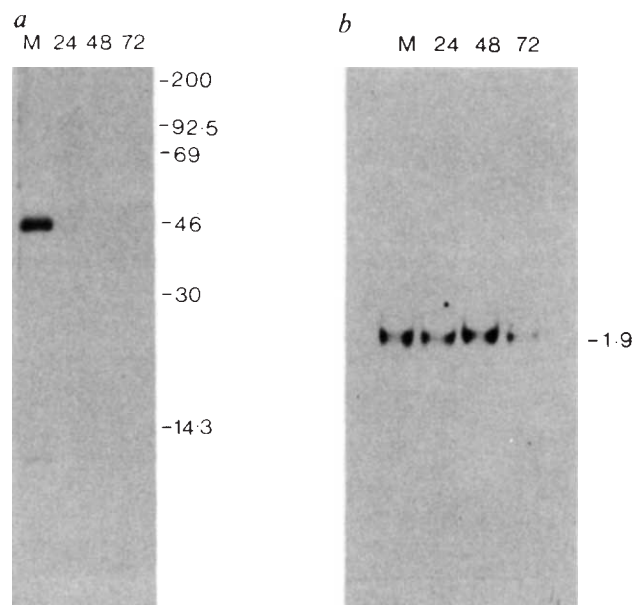


FIG. 3 *a*, Synthesis of mature cellular class I molecules in HCMV-infected cells analysed by immunoprecipitation with W6/32, a monoclonal antibody that reacts with complexed human class I molecules, but not with dissociated heavy chains<sup>18</sup>. M, mock-infected Flow 2002 cells. The numbers 24, 48, 72 refer to the time in hours after infection with HCMV at which cells were labelled with [<sup>35</sup>S]methionine. Relative molecular mass markers (in K) are shown to the right. *b*, Northern blot of RNA extracted from HCMV-infected cells and mock-infected cells (M) hybridized to a human class I probe. RNA was extracted from infected cells at 24, 48 and 72 h after infection. RNA size (kb) is shown to the right.

**METHODS.** *a*, Monolayers of Flow 2002 cells were infected with HCMV strain AD169 at a m.o.i. of five. Infected cells were labelled with [<sup>35</sup>S]methionine for 4 h at 24, 48 and 72 h after infection and were collected after the labelling period. Mock-infected cells (M) were also labelled for 4 h. Lysate preparations and immunoprecipitations using monoclonal W6/32 were carried out as described for Fig. 1, and samples were analysed on 15% SDS polyacrylamide gels followed by autoradiography. *b*, Total RNA was extracted from Flow 2002 cells that had been infected at a m.o.i. of five with HCMV strain AD169 for 24, 48 and 72 h. RNA (5  $\mu$ g) from each time point and from uninfected cells (M) was fractionated by electrophoresis in 1.5% agarose/formaldehyde gels and transferred to nitrocellulose. Filters were hybridized in 5 $\times$ SSC buffer, 50% formamide at 42  $^{\circ}$ C to a [<sup>32</sup>P]-labelled 1.4-kb cDNA of the HLA-A2.2Y gene<sup>19</sup>. The filter was washed in 0.1 $\times$ SSC at 60  $^{\circ}$ C, air-dried and exposed to X-ray film.



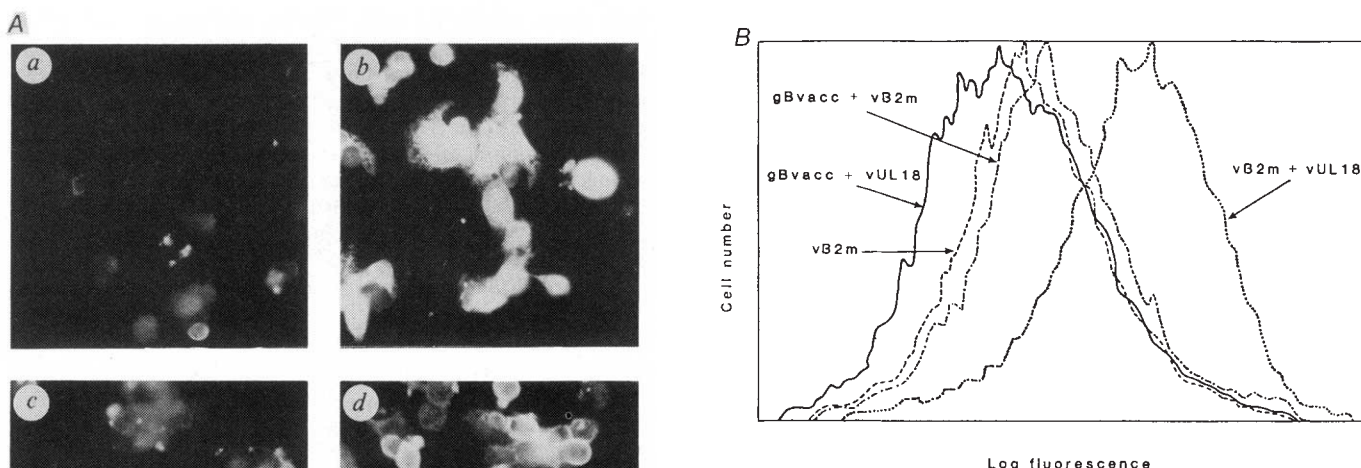


FIG. 2 A, Cellular location of  $\beta 2m$  in cells infected with recombinant vaccinia viruses as visualized by indirect immunofluorescence using monoclonal antibody BBM-1. a, Cells infected with vB2m, non-permeabilized; b, Cells infected with vB2m, permeabilized; c, cells infected with gBvacc and vB2m, non-permeabilized; d, cells infected with vB2m and vUL18, non-permeabilized. B, Surface expression of  $\beta 2m$  on cells infected with vaccinia recombinants measured by flow cytometry.

**METHODS.** For indirect immunofluorescence, BHK cells seeded on glass coverslips were infected for 16 h with recombinant vaccinia viruses at a m.o.i. of 30 for single infections and of 15 of each virus for double infections. Infected cells were fixed in 2% formaldehyde, 1% fetal calf serum in phosphate buffered saline (PBS). For internal staining, cells were permeabil-

ized with 1% Triton X100 in PBS, 1% fetal calf serum, and non-specific binding of antibody was blocked with PBS containing 1% fetal calf serum, 1% bovine serum albumin, 1% normal rabbit serum (PBS/FCS/BSA/NRS). After incubation with monoclonal antibody BBM1, specific antibody binding was detected with fluorescein-labelled rabbit anti-mouse immunoglobulin, and fluorescence was observed with ultraviolet illumination at magnification  $\times 400$ . For quantitative analysis of surface fluorescence, monolayers of BHK cells were infected with recombinant viruses for 16 h. Infected cells were scraped into PBS/FCS/BSA/NRS, pelleted at 500g for 3 min and resuspended in PBS/FCS/BSA/NRS. Monoclonal antibody BBM1 was added to the cell suspension, which was then incubated for 30 min with rotation. Cells were pelleted and washed in PBS/FCS/BSA/NRS 3 times before incubation for 30 min with fluorescein-labelled rabbit anti-mouse immunoglobulin at a dilution of 1 in 80. After a further three washes as above, cells were resuspended in 0.5 ml PBS containing 1% BSA and fixed by adding an equal volume of PBS containing 2% formaldehyde, 1% BSA. Surface fluorescence was analysed using an Epics-Profile flow cytometer (Coulter Electronics) and the single-cell population was identified by forward and 90° scatter. The data are presented as smoothed frequency histograms in which the histograms are normalized to identical peak values to allow convenient comparison of the different distributions.

Stannard reported that  $\beta 2m$  attaches to components in the virion tegument and not to the surface of enveloped particles, a result entirely inconsistent with the view that  $\beta 2m$  attaches to a transmembrane class I homologue.

Class I molecules associate with degraded endogenous proteins and present these peptides at the cell surface<sup>9,10</sup>, and in virus-infected cells, class I antigen presentation permits recognition of viral antigen by autologous lymphocytes carrying the CD8 antigen. Several viruses have been shown to evade immune recognition by interfering with class I-mediated presentation<sup>11,12</sup> and it is tempting to suppose that the role of the HCMV class I homologue is to disrupt the synthesis of mature cellular class I molecules by sequestering endogenous  $\beta 2m$ . In support of this proposal we find that in HCMV-infected cells there is no change in the overall pattern of cellular protein synthesis, but synthesis of mature cellular class I substantially decreases, whereas levels of class I heavy chain mRNA are unaltered (Fig. 3). We predict that heavy chain continues to be synthesized in these cells, but does not associate with  $\beta 2m$ , a prediction we have been unable to test directly because we have been unable to obtain an antibody that will precipitate dissociated heavy chains in cell lines permissive for HCMV. The shut down of mature class I synthesis in HCMV-infected cells would account for the fact that the main cytotoxic T cell target in these cells is a protein made immediately after infection and proteins made later in infection are poor targets<sup>13</sup>. A somewhat similar situation is seen in cells infected with adenovirus type 2 (ref. 11), where the viral E3/19K glycoprotein binds to and inhibits the matur-

ation of class I molecules. Recently, murine cytomegalovirus has been shown to evade cytotoxic T cell recognition<sup>12</sup>, but in this instance the mechanism seems to be antigen-specific and must be different from that proposed here for HCMV. Nevertheless, it appears that interference with antigen presentation by means of direct protein interactions with the molecules of the MHC is a common strategy by which viruses evade immune surveillance and control. □

Received 12 March; accepted 29 August 1990.

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**ACKNOWLEDGEMENTS.** We thank B. Barrell, N. Holmes and L. Borysiewicz for helpful discussions. The work was supported by the Medical Research Council, UK.

# Identification of the primary gene defect at the cytochrome $P_{450}$ *CYP2D6* locus

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THE mammalian cytochrome  $P_{450}$ -dependent monooxygenase system is involved in the metabolism of drugs and chemical carcinogens<sup>1-5</sup>. The role of these enzymes in toxicological response is exemplified by an autosomal recessive polymorphism at the cytochrome  $P_{450}$  *CYP2D6* debrisoquine hydroxylase locus which results in the severely compromised metabolism of at least 25 drugs, and which in some cases can lead to life-threatening side-effects<sup>3,6-12</sup>. In addition, this polymorphism, which affects 8-10% of the caucasian population, has been associated with altered susceptibility to lung and bladder cancer<sup>13-16</sup>. Here we report the identification of the primary mutation responsible for this metabolic defect and the development of a simple DNA-based genetic assay to allow both the identification of most individuals at risk of drug side-effects and clarification of the conflicting reports on the association of this polymorphism with cancer susceptibility<sup>13-18</sup>.

These conflicting reports could possibly be explained by the limitations of the phenotyping assay used to identify affected

individuals (sometimes termed poor metabolizers). To determine mutation sites suitable for the development of a DNA-based genetic assay for the poor metabolizer phenotype, and to gain insights into the molecular basis of this genetic polymorphism, we isolated complementary DNAs previously associated with normal (*db1*) and mutant *CYP2D6* alleles; termed 'a' and 'b' variants<sup>11</sup> (Fig. 1). A comparison of our 'a' variant sequence (pMP33/pMP32) with the partial sequence reported previously showed our variant 'a' was correctly spliced over intron 5. This indicates that aberrant splicing over this region, proposed previously as a mechanism of the polymorphism, had not occurred

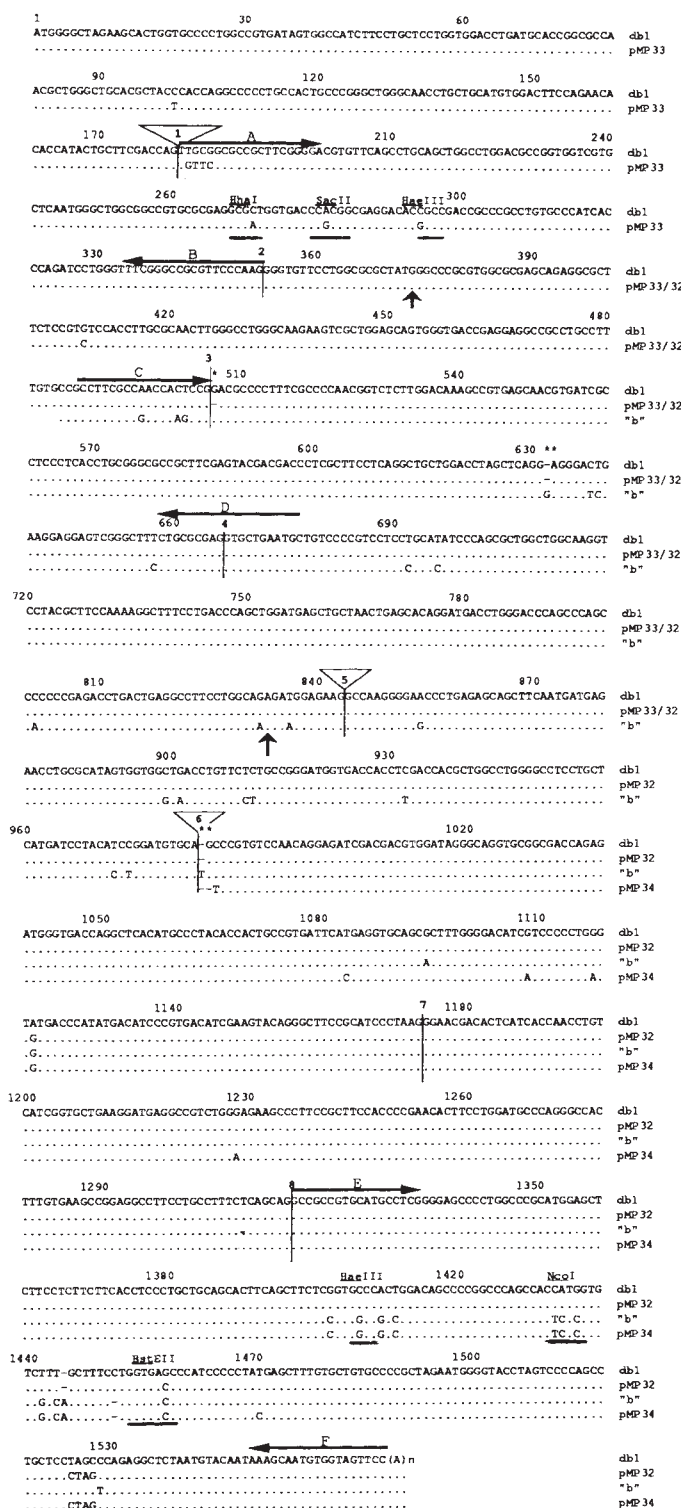
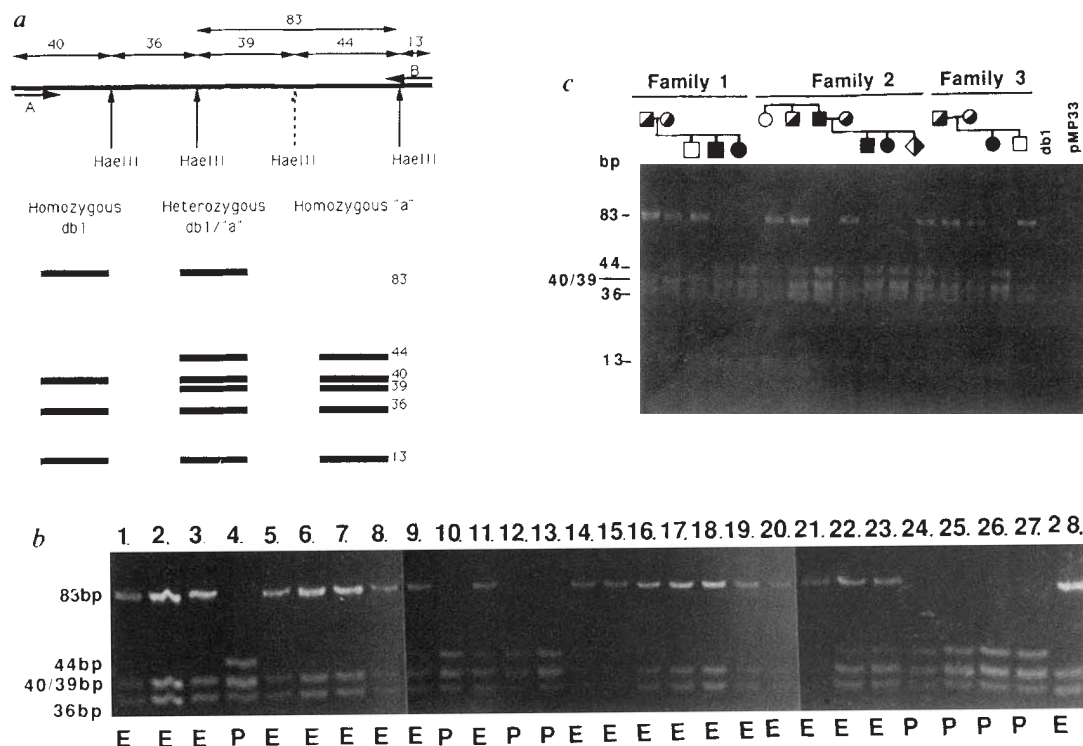


FIG. 1 Comparison of nucleotide sequences of DNA encoding functional debrisoquine hydroxylase (*db1*) and related cDNAs. The sequence for *db1* and variant 'b' are taken from Gonzalez *et al.*<sup>11</sup>. Numbering starts at the initiation codon for *db1*. The variant 'a' cDNA sequence was compiled from two cDNAs, pMP32 and pMP33 which contained an identical sequence over an overlapping region of 462 bp. This sequence contained only one base-pair difference to the partial sequence described as variant 'a' by Gonzalez *et al.*<sup>11</sup> (G to C at position 383) which covers the region 299-1,567 bp. The base-pair deletion at position 506 is marked \*. The position of introns (vertical lines) is obtained from the sequence of P450IID6 gene<sup>19</sup>. Triangles represent the positions of intron sequences in the isolated variant cDNAs: 1, the first 64-bp of intron 1 in pMP33; 5, insertion of intron 5 in the variant 'a' cDNA described by Gonzalez *et al.* but which was absent from pMP32 and pMP33; 6, insertion of part of intron 6 in both pMP34 and variant 'b'. The position of the bp insertions (G) at position 631 and at 983 (T) in the 'b' variant are shown: \*\*. The positions of oligonucleotide primers used to amplify specific regions of the genes are marked by capital letters and horizontal arrows. The oligonucleotide marked D was taken from the sequence of intron 4 (see Fig. 3). The diagnostic restriction sites used to differentiate between either *db1* (variant 'a') and variant 'b' or *db1* and variant 'a' are marked.

METHODS. Restriction fragments for use as probes were isolated from low gelling temperature agarose and radioactively labelled with [ $\alpha$ -<sup>32</sup>P]dCTP (3,000 Ci mmol<sup>-1</sup>; Amersham) by random primer extension<sup>22</sup>. The full-length P450IID6 (*db1*) cDNA was used to screen plaques from two human liver  $\lambda$ gt11 cDNA libraries (Clontech, Palo Alto, CA; and Kwok *et al.*<sup>23</sup>). Three of the isolated cDNA clones were pMP32 and pMP33 from the Clontech library; and pMP34 from the library of Kwok *et al.* were further characterized. DNA sequences were determined using the dideoxy chain termination method using [ $\alpha$ -<sup>35</sup>S]thio-dATP (400 Ci mmol<sup>-1</sup>; Amersham)<sup>24,25</sup>. Overlapping sequences were derived using a series of synthetic oligonucleotides and both DNA strands were fully sequenced. Sequences were compiled and analysed using Staden Plus software implemented on a DCS286 computer<sup>26</sup>.



FIG. 2 Association of the 'a' variant sequence at position 294 with the poor metabolizer phenotype. *a*, Diagrammatic representation of the method used. Genomic DNA across exon 2 was amplified using the primers A and B (Fig. 1). The derived fragment was then digested with *Hae*III. As a result of the C to G transversion at position 294 (hatched arrow) the 83 bp fragment in *db1* will cut into two fragments of 44 bp and 39 bp in the case of variant 'a'. The expected fragment patterns in individuals homozygous or heterozygous for these sequences are shown. *b*, Association of variant 'a' sequences with the poor metabolizer phenotype in random individuals. The data shows the *Hae*III analysis described above for 28 random poor metabolizers (P) and extensive metabolizers (E). In this experiment all the individuals homozygous for the variant 'a' sequence were all phenotyped as poor metabolizers. *c*, Identification of affected individuals in families using the diagnostic site at base pair 294. All poor metabolizers could be identified using the procedure described in Figs 2 and 3. In families 2 and 3 obligate heterozygotes could also be identified. This was not possible in family 1 as the mutant allele of the paternal obligate heterozygote appears to be due to a gene deletion. METHODS. 41 out of 42 and 30 out of 44 individuals were phenotyped as P or E by measuring the *in vivo* metabolism of the marker drugs debrisoquine or sparteine<sup>6,7</sup>. In the other cases, liver samples were phenotyped *in vitro* (samples 10, 11 and 13) by western blot analysis using an antibody to P450IID6 and in most cases by also measuring bufuralol hydroxylase activity<sup>27</sup>. DNA was isolated from whole blood, lymphocytes or human liver using an Applied Biosystems 340A nucleic-acids extractor according to the



manufacturer's instructions. Target DNA (approximately 1 µg for genomic DNA or 1 ng for cloned cDNA) was amplified using PCR (ref. 28) with 300–600 ng of each amplification primer (A and B, Fig. 1). PCR was carried out using 2.5 U *Taq* DNA polymerase (Cetus Corporation) according to the manufacturer's instructions, except that dimethyl sulphoxide was added to 10% (v/v) final concentration. DNA strands were initially separated by heating at 94° for 5 min. Subsequent steps were: primer annealing 60° (1 min), polymerization 72° (2 min) and strand separation 94° (1 min). 20–30 PCR cycles were carried out. The annealing temperature used was critical so that the P450IID6 and P450IID7 genes could be distinguished<sup>19</sup>. Between one fifth and one tenth of the amplification product was then digested with *Hae*III and the fragments separated electrophoretically on a 6% polyacrylamide gel and visualized by ethidium bromide staining.

here. Also, and more importantly, the variant 'a' sequence contained a base-pair (G) deletion at position 506. This deletion introduces a frameshift and leads to a stop codon at position 544. A functional *P*<sub>450</sub> protein therefore could not be produced. This base-pair deletion was also present in the variant 'a' sequence reported previously but no reference was made to it<sup>11</sup>.

The other cDNA clone (pMP34) which was very similar to that described as variant 'b' would not encode a functional *P*<sub>450</sub> because of an insertion which seems to be part of intron 6 (Fig. 1). There are also 1-base pair (bp) insertions in variant 'b' in exons 4 and 7 at position 631 and 983 which cause frame-shifts and break the open reading frame.

To establish whether variants 'a' and 'b' represent mutant alleles of the *db1* gene and to develop a DNA-based genetic assay we looked to see whether the base-pair differences between them and *db1* are informative for the poor metabolizer phenotype. The base-pair differences between the functional *db1* sequence and variants 'a' and 'b' in most cases results in the acquisition, or loss, of a restriction site. Amplification of genomic DNA over these sites, followed by digestion with the appropriate restriction enzyme, will therefore establish whether individuals carry the *db1* or 'a' or 'b' variant sequences.

Analysis of variant 'b' was carried out by amplifying a 251-bp region of exon 9 using polymerase chain reaction (PCR) primers E and F followed by digestion with *Nco*I which will only cut the *db1* and variant 'a' PCR fragment and not the variant 'b'

fragments (Fig. 1). Of 40 samples studied, 38 contained both *db1* (variant 'a') and variant 'b' sequences (not shown). Variant 'b' is therefore not associated with the poor metabolizer phenotype as previously described, but is the product of a different gene from *db1*.

In these experiments in two individuals no *db1* (or variant 'a') PCR amplification product was obtained. One individual was a phenotyped poor metabolizer, the other was of unknown phenotype. Subsequent analysis of these samples using PCR primers A+B and C+D (Fig. 1) indicated that in both cases all the *db1* gene was deleted. This homozygous deletion has only been found in 1 out of 42 phenotyped poor metabolizer individuals and is therefore an uncommon mutant allele. The individual with this homozygous deletion was also homozygous for the 11.5-kilobase (kb) *Xba*I restriction fragment length polymorphism (RFLP) informative for the poor metabolizer phenotype. The allele frequency of this 11.5-kb RFLP is approximately 13–16% in the affected and ~1–2% in the total population<sup>20,21</sup>. Recent sequencing studies on DNA clones derived from an individual homozygous for the 11.5-kb restriction fragment confirm the presence of a P450IID6 gene deletion<sup>21</sup>.

The similarity of the sequence of variant 'a' with the known functional *db1* sequence and the fact that neither *db1* nor variant 'a' sequences could be detected in the poor metabolizer carrying the homozygous deletion, strongly indicated that variant 'a' is an allele of the *db1* gene. We therefore analysed the base-pair

differences between *db1* and variant 'a' at positions 271 (C to A), 281 (A to G), 294 (C to G) and 506 (G deletion) for linkage with the affected phenotype. The PCR primers and the diagnostic restriction enzymes used are shown in Fig. 1. Analysis of the site at position 294 showed close association with the affected phenotype (Fig. 2a, b). This was confirmed by using a series of families containing affected individuals (Fig. 2c). We have now studied 42 poor metabolizers and 44 extensive normal metabolizers and were able to predict the phenotype of all of the normal and 81% (34 out of 42) of the poor metabolizers. This represents a significant improvement on the RFLP-based assay described by Skoda *et al.*<sup>20</sup> which can predict 21–25% of poor metabolizers. The other two mutation sites in exon 2 at 271 and 281 were equally informative and identified the same group of poor metabolizers (data not shown).

The base-pair differences between *db1* and variant 'a' at 271 positions, 281 and 294 cannot be responsible for the affected phenotype as they are either silent, or lead to single amino-acid substitutions, and would not prevent the synthesis of *db1* protein. In this regard, however, the inactivating base-pair deletion at position 506, resulting in a frameshift described above, was of

particular interest. As a consequence of this change, a *Bst*NI restriction site present in *db1* is lost in variant 'a'. Analysis of this site (Fig. 3) showed that 79% of affected and no normal individuals were homozygous for the variant 'a' sequence and, interestingly, were the same individuals as those with the mutation sites at nucleotides 294, 281 and 271. The mutation leading to this frameshift therefore seems to be the predominant reason for the inactivation of the *db1* gene.

Examination of the *db1* sequence over the intron 3–exon 4 junction suggests two explanations for the base-pair deletion in the cDNA sequences. The *db1* intron 3–exon 4 junction has the sequence CCCCCAG/GACGCC (the bold letters indicate the start of exon 4)<sup>19</sup>. Therefore, either a base-pair (G) deletion to give CCCCCAG/ACGCC, or a G to A transition to give the sequence CCCCCAAG/ACGCC, which shifts the position of the 3' splice site, will result in the loss of the first base (G) in exon 4. In both cases the *Bst*NI restriction site is lost. To establish which was the case we sequenced the PCR amplification product from 20 affected individuals. In all of these the G to A transition was shown to be the mutation responsible for the poor metabolizer phenotype.

Reports on the association of the *CYP2D6* polymorphism and susceptibility to cancer have been either equivocal or unconfirmed. The development of a DNA-based genetic assay to predict the poor metabolizer phenotype will now allow definitive studies. We have looked at the association of this polymorphism with susceptibility to bladder and lung cancer. The incidence of both diseases is associated with cigarette smoking. Using a standard two-tailed  $\chi$ -squared analysis, out of 140 people with lung cancer, there was a significant reduction (relative to a random control population of 90 caucasian individuals) in the number of poor metabolizers ( $\chi$ -squared = 10.66 with 2 degrees of freedom,  $P < 0.005$ ). Indeed, only three individuals (instead of a predicted ten) were identified as poor metabolizers. All three were smokers; two had squamous cell carcinoma and one adenocarcinoma. On the basis of the data available it was not possible to relate tumour type and the poor metabolizer frequency. Out of 117 patients with bladder cancer there was also a significant reduction in the number of poor metabolizers, three out of a predicted eight ( $\chi$ -squared = 6.68 with 2 degrees of freedom,  $P < 0.05$ ). These preliminary data support reports<sup>13,19</sup> that the polymorphism at the *CYP2D* locus is associated with susceptibility to these diseases. It is, however, important that more samples are studied to establish clearly whether this is indeed the case. If the association proves to be real it would indicate that the protein encoded by *CYP2D6* is involved in the activation of the chemical carcinogens contained in cigarette smoke and is directly involved in the initiation of cancer. □

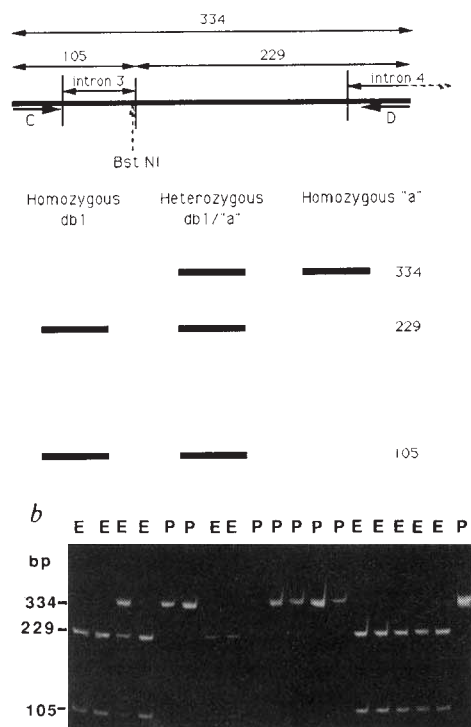


FIG. 3 Analysis of the mutation site leading to the base-pair deletion in *db1* cDNA at position 506. *a*, Diagrammatic representation of the method used and predicted banding pattern for individuals containing the *db1* gene, variant 'a' or both. Amplification of DNA from exon 3 into intron 4 produced a 334-bp fragment which in individuals containing the *db1* sequence digests into fragments of 105 bp and 229 bp with *Bst*NI. The variant 'a' sequence is resistant to digestion with this enzyme. *b*, Association between the mutation leading to the base-pair deletion at position 506 in the variant 'a' cDNA and the poor metabolizer phenotype in a series of 19 random individuals phenotyped as either poor (P) or normal (E) metabolizers. In these samples the affected phenotype segregated with individuals homozygous for the variant 'a' sequence.

**METHODS.** Genomic DNA from individuals phenotyped either as poor or normal metabolizers was amplified by PCR using an oligonucleotide derived from exon 3 and one derived from intron 4 (marked C and D in Fig. 1) using an annealing temperature of 60 °C. The sequence of oligonucleotide D was AAATCTGCTCTTCGAGGC. The use of this oligonucleotide pair and the high annealing temperature assured specificity for the P450IID6 gene. The resulting fragment of 334 bp was then digested with the restriction enzyme *Bst*NI and the products separated on an 8% polyacrylamide gel. Bands were visualized by ultraviolet irradiation of the gel stained with ethidium bromide.

Received 5 March; accepted 10 September 1990.

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ACKNOWLEDGEMENTS. We thank J. Sweeney and A. McLaren for their help in sequencing the PCR products; F. Gonzalez for providing the *db1* cDNA; U. Meyer for a monoclonal antibody used to phenotype a series of liver samples and for phenotyping several of these; G. Tucker for samples from *in vivo* phenotyped individuals; A. Murday and T. Bishop for blood samples; W. Bodmer, N. Hastie for critical comments, and R. Wood for help with the statistical analysis. DNA sequences generated during the course of this work have been deposited in the EMBL Data Bank with accession nos. X16865, X16866 and X16867.

## X-ray analysis of $\beta$ B2-crystallin and evolution of oligomeric lens proteins

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THE  $\beta$ ,  $\gamma$ -crystallins form a class of homologous proteins in the eye lens. Each  $\gamma$ -crystallin comprises four topologically equivalent, Greek key motifs; pairs of motifs are organized around a local dyad to give domains and two similar domains are in turn related by a further local dyad<sup>1–4</sup>. Sequence comparisons and model building predicted that hetero-oligomeric  $\beta$ -crystallins also had internally quadruplicated subunits, but with extensions at the N and C termini, indicating that  $\beta$ ,  $\gamma$ -crystallins evolved in two duplication steps from an ancestral protein folded as a Greek key<sup>5–7</sup>. We report here the X-ray analysis at 2.1 Å resolution of  $\beta$ B2-crystallin homodimer which shows that the connecting peptide is extended and the two domains separated in a way quite unlike  $\gamma$ -crystallin. Domain interactions analogous to those within monomeric  $\gamma$ -crystallin are intermolecular and related by a crystallographic dyad in the  $\beta$ B2-crystallin dimer. This shows how oligomers can evolve by conserving an interface rather than connectivity. A further interaction between dimers suggests a model for more complex aggregates of  $\beta$ -crystallin in the lens.

Beta-crystallins comprise a multigene family of basic [ $\beta$ B1,  $\beta$ B2,  $\beta$ B3] and acidic [ $\beta$ A1,  $\beta$ A2,  $\beta$ A3,  $\beta$ A4] polypeptides<sup>8</sup>. The oligomeric bovine  $\beta$ -crystallin sequences have between 45% and 60% identity with each other for the regions corresponding to the globular domains and about 30% identical residues with monomeric  $\gamma$ -crystallins. A single-domain protein with a crystallin-like fold has been found in *Amoeba*<sup>9</sup> and a two domain, four Greek key motif protein in *Myxococcus xanthus*<sup>10</sup>. Whereas exons encode separate motifs in  $\beta$ -crystallins, in  $\gamma$ -crystallins they correspond to complete domains<sup>6,7</sup>.  $\beta$ B2, previously known as  $\beta$ Bp, is the predominant subunit in hetero oligomers; it is versatile in its interactions being able to self-associate into dimers or interact with other acidic and basic  $\beta$ -subunits to form dimers and larger aggregates<sup>11</sup>.

$\beta$ B2-crystallin was crystallized with one subunit in the asymmetric unit<sup>12</sup> and was solved by molecular replacement as described in the legend to Fig. 1. The electron density calculated on the basis of the refined structure was discontinuous around residue 86 when the connecting peptide between the domains was arranged as it is in  $\gamma$ B, previously known as  $\gamma$ -II crystallin. When, however, the connecting peptide and adjacent tripeptides were removed from the calculation of phases used in difference Fourier, continuous electron density clearly indicated the path of the polypeptide (Fig. 1). The extensions at the N and C termini are in less clear density, indicating their greater flexibility

FIG. 1 Analysis and electron density. A stereo view of a section of the refined electron density map (contoured at  $1\sigma$ ) and superposed atomic model of the connecting peptide region of  $\beta$ B2 between residues 84 and 88 with the side chain of symmetry-related Gln 86 nearby.

METHODS. X-ray data collected on the Synchrotron Radiation Source at SERC Daresbury were merged to give 10,510 unique reflections at 2.65 Å with  $R_{\text{sym}}$  of 3.9%.  $\gamma$ B refined at 1.5 Å resolution<sup>2</sup> was used as a search molecule in molecular replacement. Initially the  $\gamma$ B molecule was aligned with its interdomain pseudo twofold axis parallel to the  $z$  axis of a P1 cell of dimensions  $80 \times 50 \times 50$  Å. Rotation function runs (using Crowther's fast rotation function), carried out with Patterson radii between 6 and 18 Å and using  $E$  values in the resolution range 3.0–20.0 Å, yielded two peaks  $\sim 180^\circ$  apart in  $\gamma$  corresponding to the solution and the twofold related pseudosolution. Use of the translation function completed the location of the molecule and indicated the correct space group as I222; the 10 highest peaks for  $I2_12_12_1$  all gave rise to molecular packings with unacceptable overlaps. Rigid body refinement yielded an  $R$ -factor of 45.1%. Cycles of simulated annealing as implemented in XPLOR<sup>16</sup> together with restrained least squares refinement using RESTRAIN and manual model building as described previously<sup>4</sup>, decreased the agreement value to 0.19. At this stage there were discontinuities in the connecting peptide and lack of clear density at the insertion 70A and 71B. Residues 79 to 91 were removed and the coordinates were randomized (up to  $\pm 0.5$  Å) to remove the model bias. After subsequent refinement using RESTRAIN, a difference map using  $(|F_{\text{obs}}| - |F_{\text{calc}}|)$  coefficients was calculated. The chain was built into the electron density which clearly indicated that the domains were connected differently from  $\gamma$  and a different asymmetric unit was chosen. The model was refined and a new map with the coefficients  $(2|F_{\text{obs}}| - |F_{\text{calc}}|)$  was calculated. The sequence extensions at the N and C termini were modelled into relatively weak density. A second native data set was collected at the SRS to give some 18,000 unique reflections at 2.1 Å with  $R_{\text{sym}}$  of 6.3%. To test the unexpected connectivity of the connecting peptide around the twofold axis with the independent higher resolution data set, three experiments were done. First, the connecting peptide residues 84–87 were left out, as were the N- and C-terminal arms, and the two domains randomized by  $\pm 0.5$  Å to destroy any coordinate memory. The coordinates were kept in the  $\beta$ B2 asymmetric unit. XPLOR energy minimization was performed, initially keeping the C $\alpha$ -atoms fixed; the coordinates were then heated to 2,000 K and slowly cooled. The resulting  $(2|F_{\text{obs}}| - |F_{\text{calc}}|)$  electron density map clearly confirmed the connectivity. In the second experiment the  $\gamma$ B-type connecting peptide was built with the main chain occupying the density of what constitutes in the final model the side chain of His 88. No arms were included and the insertion region 70–72 was rebuilt using density from the first experiment. The connecting peptide region was highly strained. The coordinates without randomization were put in a  $\gamma$ B asymmetric unit and XPLOR was performed as in the first experiment. Again the  $(2|F_{\text{obs}}| - |F_{\text{calc}}|)$  electron density map showed the presence of the  $\beta$ B2 connecting peptide and the  $(|F_{\text{obs}}| - |F_{\text{calc}}|)$  map showed the incorrectness of the rebuilt  $\gamma$ B-like model. In the third experiment the coordinates were refined into the 2.1 Å data with two passes of RESTRAIN refinement and model building. No arms or insertion were present after the first pass. Then, after removing the connecting peptide residues 86–89, the coordinates (in the  $\beta$ B2 asymmetric unit) were subjected to XPLOR as described for experiment 1. The connecting peptide was fitted into the electron density map and the coordinates were refined using three passes of RESTRAIN at 2.1 Å with attempts to build in the arms and a better insertion. Electron density for the arms remained inconclusive. The insertion was rebuilt and after the coordinates were subjected to another pass of XPLOR at 2.1 Å, heating to 3,000 K, the final residual was 18.6%. A region of this electron density map is shown above. The coordinates will be submitted to the Brookhaven Protein Data Bank.

which is consistent with their high sequence variability and the large number of alanines and prolines in some  $\beta$ -crystallins. The resulting structure was refined to an agreement residual of 0.18 for all data between 10.0 and 2.1 Å resolution.

The two domains of a  $\beta$ B2 subunit are not in close contact but are separated by an extended connecting peptide (Fig. 2b). There are extensive interactions between the subunits analogous to those between N- and C-terminal domains within monomeric  $\gamma$ -crystallins (Fig. 2a). The N-terminal domain of subunit 1 interacts with the C-terminal domain of subunit 2, and the C-terminal domain of subunit 1 interacts with N-terminal domain of subunit 2 (Fig. 2c). The two subunits are related by a crystallographic dyad called P. The interface between the

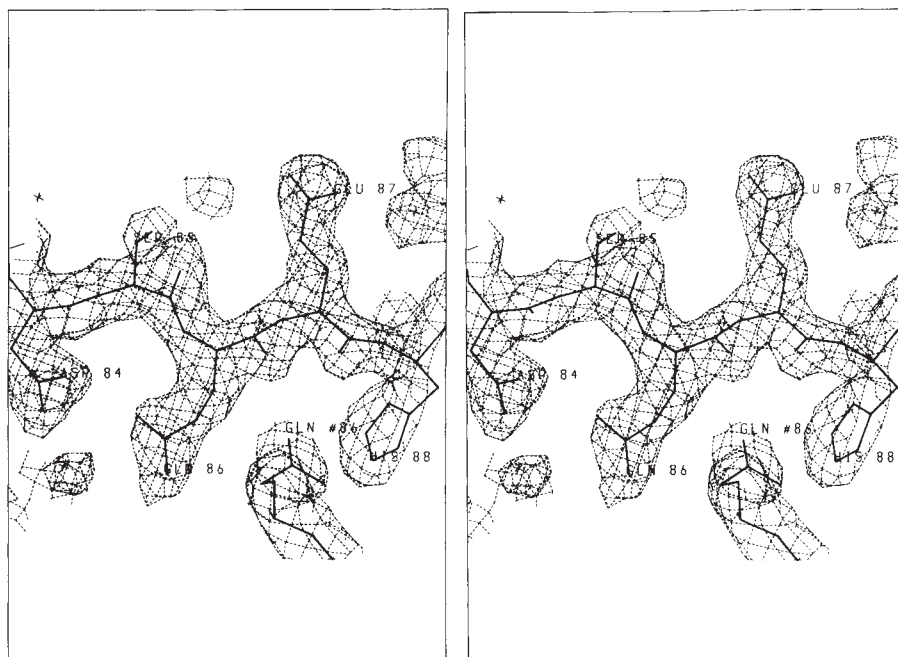
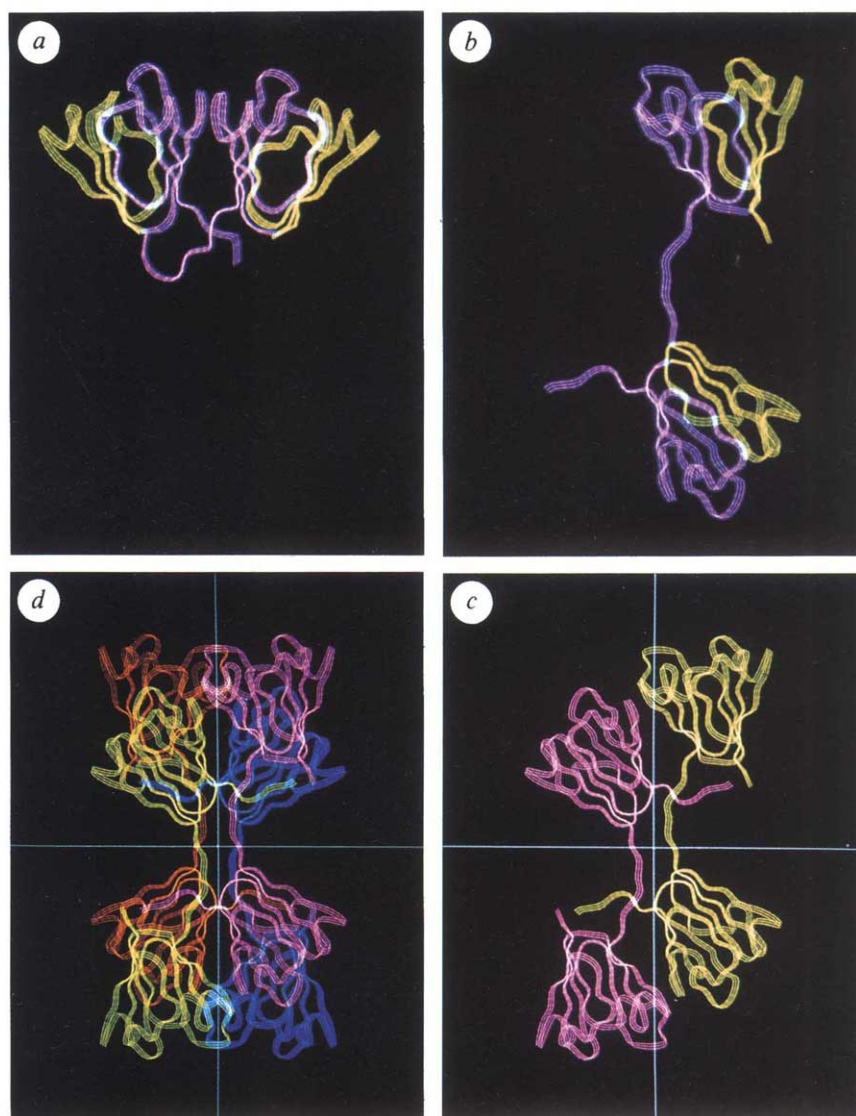


FIG. 2 Ribbon diagrams showing the path of the  $\alpha$ -carbon atoms of the polypeptide chain with definition of axes and interfaces. *a*, The  $\gamma$ B molecule is shown with motifs 1 and 3 coloured green whereas motifs 2, 4, the connecting peptide and the short C-terminal tail are coloured violet. The molecule is viewed approximately perpendicular to the pseudo twofold axis relating the N-terminal domain on the right to the C-terminal domain on the left. The interface between domains is formed from  $a b d \beta$  strands (Fig. 3) from motifs 2 and 4. *b*, One subunit of  $\beta$ B2 with the equivalent regions coloured violet as depicted in *a* for  $\gamma$ B. The N-terminal domain is in the upper and the C-terminal domain in the lower part of the diagram. *c*,  $\beta$ B2 dimer. Subunit 1, coloured green, is in the same orientation as in *b* and is related to subunit 2, coloured magenta, by a crystallographic twofold P axis perpendicular to the page. The vertical line denotes Q and the horizontal line the R axis. The N- and C-terminal domains from the two different subunits interact at the PQ interface whereas there is little contact between domains at the PR interface. *d*,  $\beta$ B2 tetramer. A further dimer, comprised from subunit 3 (blue) related by the P axis to subunit 4 (brown), interacts with the magenta/green dimer around the crystallographic Q and R dyads to give a tetramer with 222 symmetry.





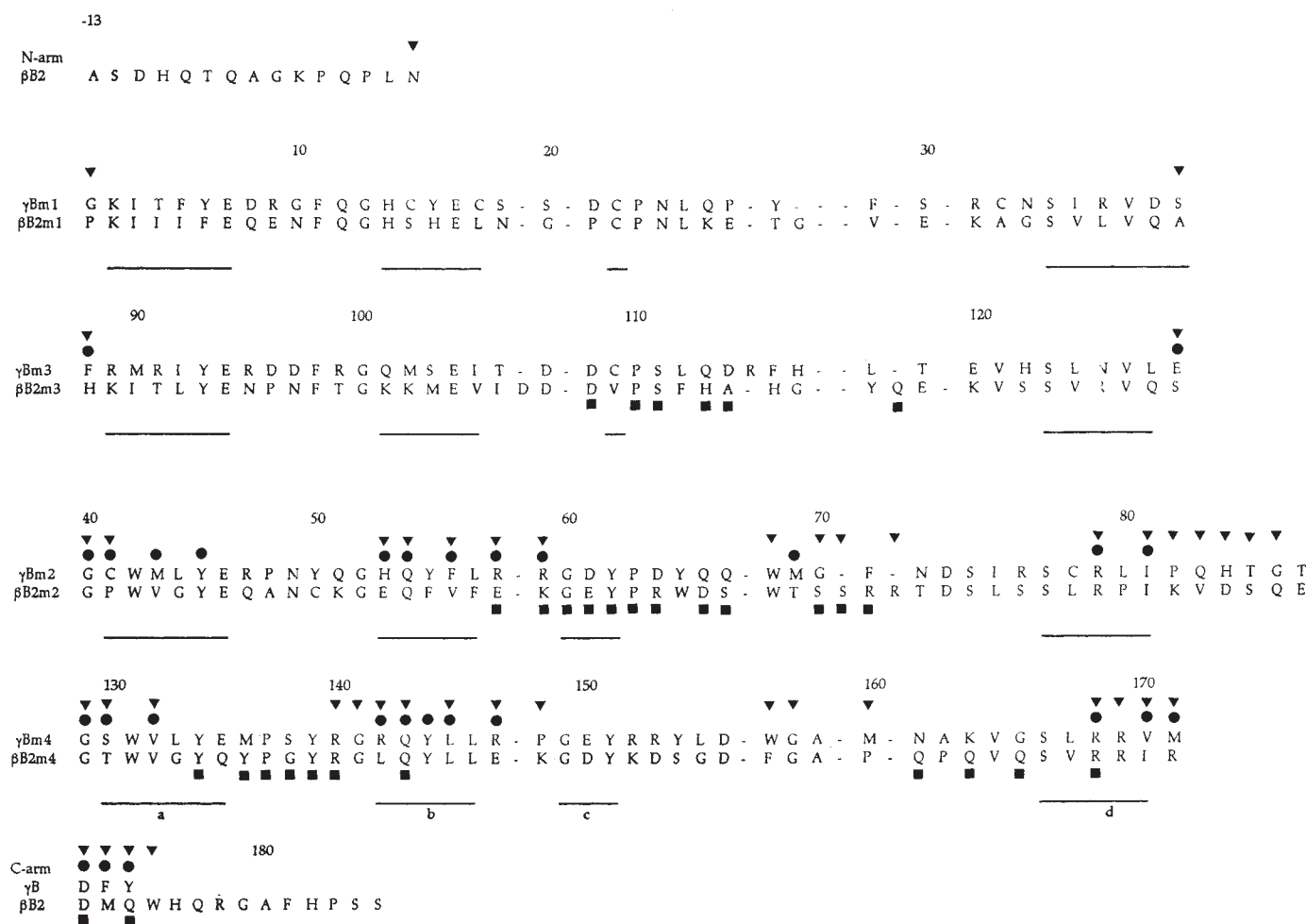


FIG. 3 Sequence alignment of the four motifs of  $\beta$ B2 and  $\gamma$ B arranged so that topologically equivalent residues are aligned in vertical rows. The numbering is based on  $\gamma$ B.  $\beta$ B2 has 15-residue N-terminal and 11-residue C-terminal extensions, additions to the globular domains at 28A, 70A, 71B, 106A, 118A and a deletion at 115 compared with  $\gamma$ B. Positions 28, 68, 116, 157 are aligned as each has a backbone H-bond with positions 25, 65, 112, 154 respectively. Also, positions 28A, 117, 158 from  $\beta$ B2 and 69,

117, 158 from  $\gamma$ B have a positive  $\phi$  torsion angle. Residues buried  $>10 \text{ \AA}^2$  in a particular interface, as measured by the decreased accessibility<sup>17</sup>, were considered to contribute towards a particular interface: ●, residues buried in the interdomain interface of  $\gamma$ B if the accessibility of the domains are measured when they are cut between residues 85 and 86 and then separated; ▼, residues buried in the PQ interface of a dimer of  $\beta$ B2; ■, residues buried in the QR interface of a dimer-dimer of  $\beta$ B2.

subunits in  $\beta$ B2-crystallin leads to a surface area, named PQ, of  $2,200 \text{ \AA}^2$  per subunit being removed from the solvent. The dimer is related to a further dimer by two orthogonal dyads Q and R, to give a tetramer with crystallographic 222 symmetry (Fig. 2d). This is achieved by burying a further contact area named QR of  $1,350 \text{ \AA}^2$  per subunit (Fig. 3).

The dimer is stabilized at the PQ interface by interactions between  $\beta$  sheets mainly from motif 2 and motif 4, which pack against each other (Figs 2c and 3). In addition, Val 83 at the end and Pro 1 at the start of the N-terminal domain interact with Met 173 and Trp 175 respectively at the end of the C-terminal domain. The dimers are also stabilized by numerous hydrogen bonds which are largely between main chain and side chains. In the connecting peptide the side chain of Asp 84 forms a charged hydrogen bond with the main chain amide of residue 129 of the other subunit. The imidazole of His 88 also forms a hydrogen bond with Asp 106A, which is characteristic of basic  $\beta$ -crystallins and seems to fix the orientation of the imidazole.

Small differences in the PQ interfaces in  $\beta$ - and  $\gamma$ -crystallins may derive from all betas having proline at residue 80 at the end of the d strand of motif 2 whereas all gammas have proline at residue 82 (Figs 4d and 3). In addition, the hydrophobics on the a and b strands (Val 43 and Val 56) are smaller in all  $\beta$ -crystallins than in  $\gamma$ -crystallins. Arginines in  $\gamma$ -crystallins (58

and 147) at equivalent positions in the N- and C-terminal domains are replaced with glutamates in  $\beta$ -crystallins which make ion pairs near the local dyad. These differences between  $\beta$ - and  $\gamma$ -crystallins are unlikely to be responsible for the preference for dimers in  $\beta$ -crystallins as they would be identical in either arrangement. There is a larger number of intermolecular contacts between domains in the  $\beta$ -crystallins compared with intramolecular contacts in  $\gamma$ -crystallins. Many of these are, however, due to an insertion in the  $\beta$ -subunit which contributes to the interface in such a way that either a monomeric or dimeric  $\beta$ -crystallin arrangement would be equally stabilized. The major structural difference most probably arises from the sequences of the connecting peptides.

In  $\beta$ -crystallins the connecting peptide is extended (Fig. 4a) but takes a sharp turn in  $\gamma$ -crystallins so that the two domains interact. In  $\gamma$ -crystallins this is achieved in one of two ways. In  $\gamma$ B, residue 86 is a glycine with a positive torsion angle  $\phi$  allowing a change in direction that is stabilized by the side chain of His 84 hydrogen bonding to the backbone carbonyl of residue 87 (Fig. 4b). Sometimes the connecting peptide is shorter by one residue and in this case the three central residues of the turn (84, 85, 87) always comprise those amino acids which most frequently occur in linker oligopeptides between domains<sup>13</sup> allowing some stabilization of local backbone polar atoms (Fig.

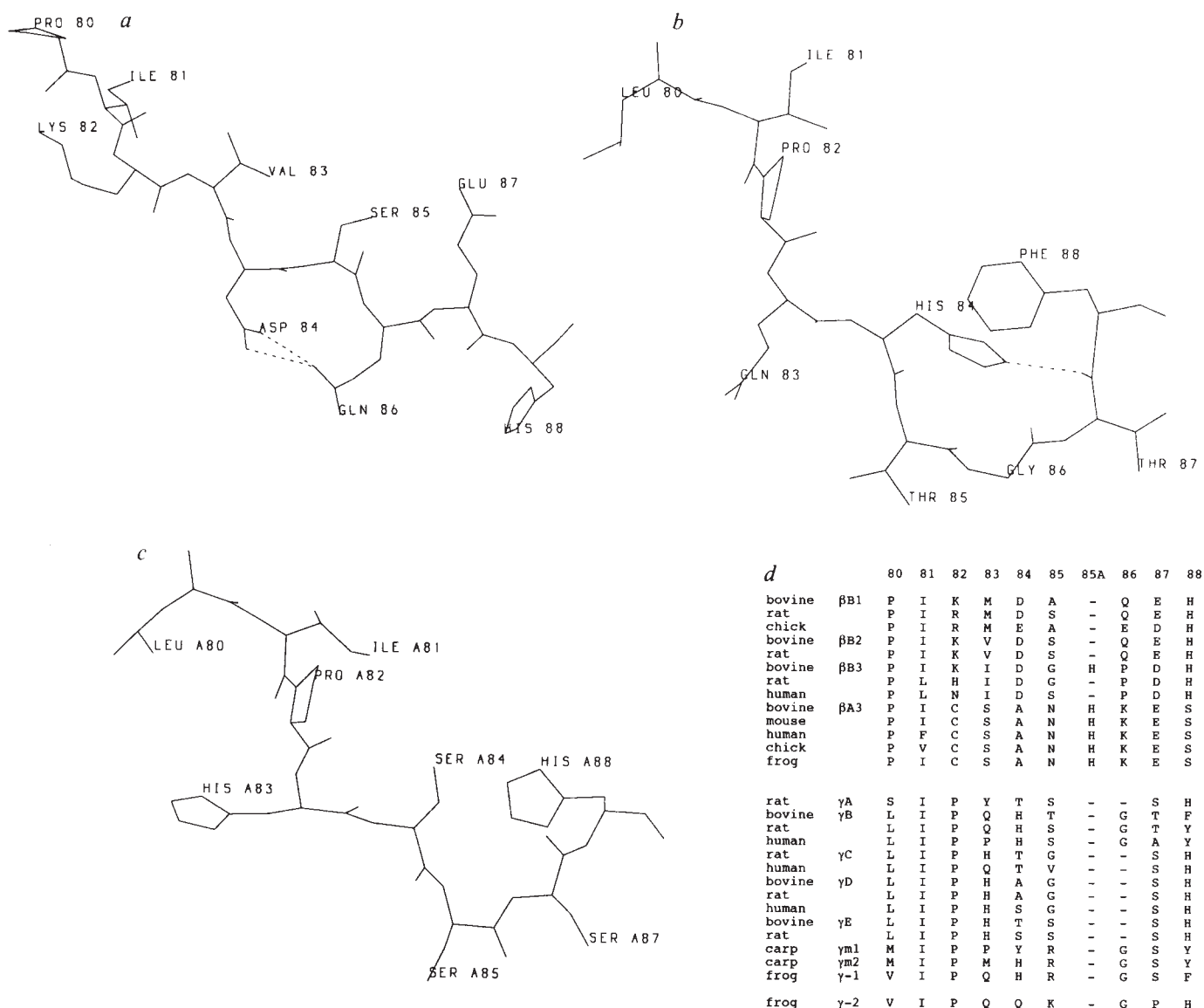


FIG. 4 Comparison of conformation and sequences of the connecting peptide regions of  $\beta$ ,  $\gamma$ -crystallins between residues 80 and 88. *a*,  $\beta$ B2 showing an extended conformation with the side chain of Gln 86 H-bonded with Asp 84. Backbone atoms N84, N86 and side chain Ser 85 interact with the other subunit of the dimer related by the twofold P axis at the side chain positions of Ser 130, His 88 and main chain O171 respectively. *b*, Bovine  $\gamma$ B showing how the backbone can change direction if residue 86 is glycine

and is stabilized by a H-bond between the side chain of His 88 and backbone O87. *c*, Rat  $\gamma$ E molecule A (H.D. and C.S., unpublished results) showing how deletion of Gly 86 still permits the turn. *d*, Alignment of sequences from bovine, rat and chick  $\beta$ B1<sup>18</sup>, bovine<sup>19</sup> and rat  $\beta$ B2<sup>20</sup>, bovine<sup>8</sup>, rat, human  $\beta$ B3<sup>21</sup>, bovine, mouse and chick  $\beta$ A3<sup>22</sup>, human  $\beta$ A3<sup>23</sup>, frog  $\beta$ A3<sup>24</sup>, rat  $\gamma$ A-F<sup>25</sup>, bovine  $\gamma$ B<sup>26</sup>, human  $\gamma$ B, C<sup>27</sup>, bovine<sup>28</sup> and human  $\gamma$ D<sup>29</sup>, bovine  $\gamma$ E<sup>4</sup>, carp  $\gamma$ m1, 2<sup>30</sup>, frog  $\gamma$ 1, 2<sup>31</sup>.

4c and *d*). There are no  $\beta$ -crystallins with short connecting peptides and none has a glycine at 86. Instead several of the residues in the connecting peptide have bulky, polar side chains with a conserved acidic residue at 87.

Thus the  $\beta$ ,  $\gamma$ -crystallin superfamily demonstrates how modification of an existing interface rather than evolution of a new one can give rise economically to novel assemblies during evolution.

The  $\beta$ B2 subunit not only self-associates to a homodimer but also readily forms heterodimers with  $\beta$ B3 and  $\beta$ A4 and a larger aggregate with  $\beta$ A3<sup>11</sup>. As both  $\beta$ - and  $\gamma$ -crystallins have used the same PQ interface for N- and C-terminal domain interactions it is reasonable to assume that heterodimers make equivalent interactions around pseudo twofold axes, especially as most of the residues involved are conserved in all  $\beta$ -crystallins. The crystal structure of the  $\beta$ B2 suggests a model for the next level

of interaction. The extensive lattice contact at interface QR (Figs 2*d* and 3) indicates that at high concentration  $\beta$ B2 dimers could form tetramers. Larger  $\beta$ -crystallin aggregates are likely to be tetramers with a heterodimer-heterodimer interaction using the QR contacts.

The  $\beta$ B2 dimer is similar to two  $\gamma$ -crystallin molecules, not much in contact yet highly correlated in their relative positions (Fig. 2*a*, *c*). This kind of oligomerization may reflect the need of the lens to evolve interacting symmetrical domains for stability<sup>1</sup> while suppressing their tendency to form long range close-packed symmetrical clusters which would compromise transparency<sup>14</sup>. These observations also show that a family of multi-domain proteins has evolved through multiple gene duplications. The variety of structural and recognition functions achieved using a single protein fold is reminiscent of the immunoglobulin superfamily<sup>15</sup>. □



Received 2 July; accepted 14 August 1990.

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ACKNOWLEDGEMENTS. We are grateful to the Medical Research Council for financial support.

## The *Drosophila claret* segregation protein is a minus-end directed motor molecule

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A PRODUCT encoded at the *claret* locus in *Drosophila* is needed for normal chromosome segregation in meiosis in females and in early mitotic divisions of the embryo<sup>1,2</sup>. The predicted amino-acid sequence of the segregation protein was shown recently to be strikingly similar to *Drosophila* kinesin heavy chain<sup>3</sup>. We have expressed the *claret* segregation protein in bacteria and have found that the bacterially expressed protein has motor activity *in vitro* with several novel features. The *claret* motor is slow (4  $\mu\text{m min}^{-1}$ ), unlike either kinesin or dyneins. It has the directionality, the ability to generate torque and the sensitivity to inhibitors reported previously for dyneins. The finding of minus-end directed motor activity for a protein with sequence similarity to kinesin suggests that the dynein and kinesin motor domains are ancestrally related. The minus-end directed motor activity of the *claret* motor is consistent with a role for this protein in producing chromosome

movement along spindle microtubules during prometaphase and/or anaphase.

Mutants in the *claret* (*ca*) locus in *Drosophila* show frequent chromosome nondisjunction and loss. Molecular analysis of the locus demonstrates that *claret* eye colour is caused by mutations in a gene that is closely linked, but separate from the segregation gene<sup>4</sup>. Mutations that cause both *claret* eye colour and chromosome nondisjunction are small deficiencies that affect both genes<sup>4</sup>. Sequence analysis of complementary DNAs<sup>3,5</sup> has led to the classification of the segregation protein as a member of the kinesin family of microtubule motor proteins. The predicted amino-acid sequence of the C-terminus of the segregation protein encoded at the *claret* locus is 40–45% identical to the motor domain of the heavy chain of kinesin<sup>3,5</sup>, and includes the kinesin sequences proposed for ATP and microtubule binding. However, the putative motor domain in the *claret* protein is located in the C terminus, in contrast to the N terminus as in kinesin.

Using video-enhanced differential interference contrast (VE-DIC) microscopy<sup>6</sup>, we examined the motor activity of a bacterially expressed *claret* protein. The bacterially expressed protein is missing either 30 or 45 amino-acid residues at its N terminus, depending on whether the first or second AUG is used as the start codon. Since the motor domain is in the C terminus of the molecule<sup>3,5</sup>, the motility properties of the truncated protein are expected to resemble those of the full-length protein, as has been demonstrated for truncated kinesin proteins<sup>7</sup>.

A 5  $\mu\text{l}$  sample of a bacterial lysate containing the *claret* protein was added to a clean coverslip and allowed to adsorb to the glass surface. When taxol-stabilized microtubules and 5 mM

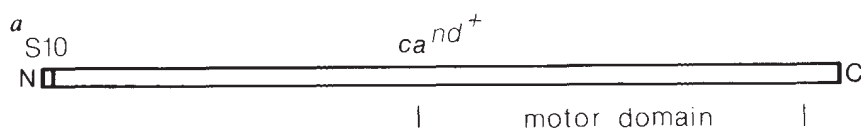
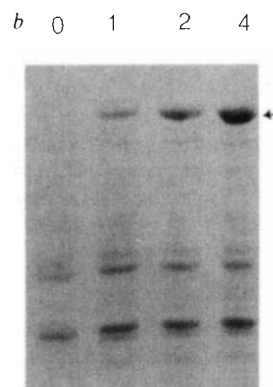


FIG. 1 Expression of *ca*<sup>nd+</sup> in bacterial cells. *a*, A *ca*<sup>nd+</sup> DNA sequence encoding an almost full-length protein was inserted into the *Bam*HI-digested and repaired plasmid pET3b (ref. 20). The *ca*<sup>nd+</sup> gene sequence encodes amino acids 46–700 of the wild-type protein<sup>3</sup>, starting from the first in-frame AUG. The putative motor domain of the protein lies between amino acids 349–671 (ref. 3). The *ca*<sup>nd+</sup> sequence is expressed as a fusion protein containing the N-terminal 11 amino acids of bacteriophage T7 S10 protein, 3 linker amino acids and 655 residues encoded by *ca*<sup>nd+</sup>. *b*, Expression of the *ca*<sup>nd+</sup> protein in *Escherichia coli*. The pET3b-*ca*<sup>nd+</sup> construct was transformed into BL21 (DE3) host cells<sup>20</sup> containing the bacteriophage T7 RNA polymerase gene under control of the *lac* UV5 promoter, which is inducible by isopropyl-beta-D-thiogalactopyranoside (IPTG). Cells were grown at 37 °C to A<sub>600</sub> = 0.6–1.0, IPTG was added to 0.4 mM and protein was induced by growth at 20 °C. Cells were pelleted from samples taken at 0, 1, 2 and 4 h, boiled 5 min in sample buffer (50 mM Tris-HCl, pH 6.8, 2 mM sodium EDTA, 1% sodium dodecylsulphate, 8% glycerol, 1% mercaptoethanol, 0.025% bromophenol blue) and protein was separated on a 10% SDS-polyacrylamide gel. The arrow indicates the band of induced protein at relative molecular mass 80,000 (*M*<sub>r</sub> 80K). The predicted molecular weight of the fusion protein is 74K. Overnight growth in the presence of IPTG resulted in low amounts of induced protein, presumably because of protein instability, and induction at 37 °C for 4 h resulted in very low motor activity. Cultures for motility tests were therefore induced for 4 h at 20 °C.



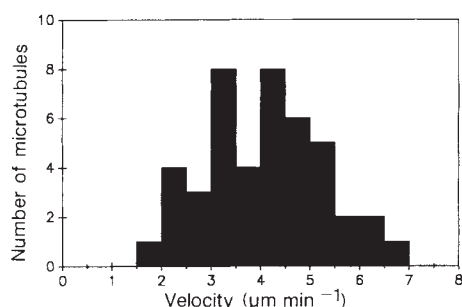


FIG. 2 Velocity histogram of microtubule gliding produced by the *claret* protein. Forty-four microtubules were measured.

**METHODS.** Lysates of induced protein were prepared essentially as described previously<sup>7</sup>. A 5-μl sample of the lysate was applied to a coverslip and augmented with magnesium ATP (final concentration, 5 mM) and taxol-stabilized microtubules in PM buffer<sup>21</sup> (final tubulin concentration, 0.04 mg ml<sup>-1</sup>) assembled from phosphocellulose-purified porcine brain tubulin<sup>21</sup>. The final volume was 9 μl. Preparations were viewed using VE-DIC microscopy as previously described<sup>6</sup>. Lysates prepared from induced cells containing pET3b with no insertion showed no motor activity.

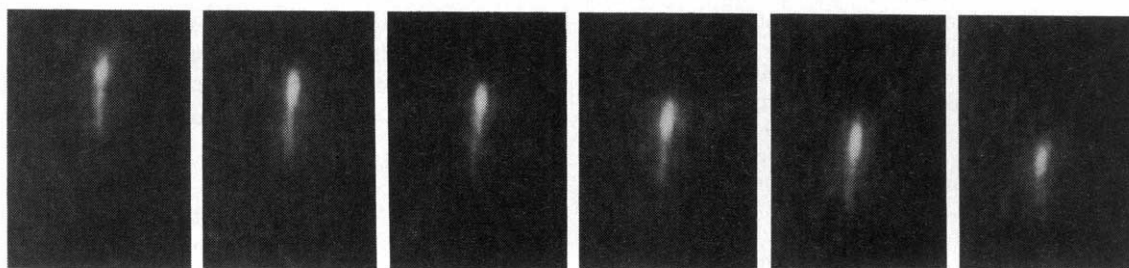


FIG. 3 Polarity of bacterially expressed *claret* motor activity. The asymmetrically-labelled fluorescent microtubule moves along the coverslip surface with the brighter, minus end trailing. The interval between each image is 10 s. The microtubule is 4.5 μm long.

**METHODS.** A mixture of 2.5 μM rhodamine-labelled tubulin<sup>16</sup>, 11.5 μM unlabelled tubulin and 14 μM *N*-ethyl maleimide-modified tubulin<sup>16</sup> was used to assemble dimly fluorescent microtubules selectively onto the plus ends of brightly fluorescent microtubule seeds crosslinked with ethylene glycol-bis-succinimidylsuccinate<sup>22</sup>, which were assembled from rhodamine tubulin. Assembly was carried out for 10–15 min at 37 °C and the resulting asymmetrical microtubules were diluted 20-fold into PM buffer containing taxol

(final taxol concentration, 5 μM). Slides were prepared as in Fig. 2, except that fluorescent microtubules were substituted for unlabelled microtubules and an anti-fade mixture<sup>15</sup> was added to limit photobleaching. Microtubule gliding was observed by time-lapse video microscopy using a Zeiss Standard microscope, IV FL illuminator, Zeiss rhodamine filter set, and a 100 W mercury arc lamp. Images were recorded using a Dage 66 SIT video camera and were stored on a Panasonic TQ2028F OMDR. As controls, we verified that axonemal dynein<sup>23</sup> moved the asymmetrically labelled fluorescent microtubules toward their plus ends whereas axonal kinesin<sup>24</sup> moved them towards their minus ends.

magnesium ATP were added to the coverslip, microtubules were observed to attach and move over the glass surface (data not shown). All attached microtubules moved and, in most cases, the entire microtubule remained attached. Thus, the bacterially expressed *claret* protein is a microtubule motor molecule that can translocate microtubules across a glass surface much like dyneins or kinesin<sup>8</sup>. Motility did not occur with 5 mM magnesium GTP, which is typical of dynein<sup>9</sup> but unlike kinesin<sup>10</sup>.

The velocity of microtubule translocation ranged from 1.3 to 6.6 μm min<sup>-1</sup>, with an average of  $4.1 \pm 1.2$  μm min<sup>-1</sup> ( $x \pm s.d.$ ,  $n = 44$ ) (Fig. 2). This is much slower than the values that have been reported for kinesin<sup>11</sup> (~30 μm min<sup>-1</sup>), cytoplasmic dynein<sup>12</sup> (~75 μm min<sup>-1</sup>), or axonemal dynein<sup>13,14</sup> (200–500 μm min<sup>-1</sup>).

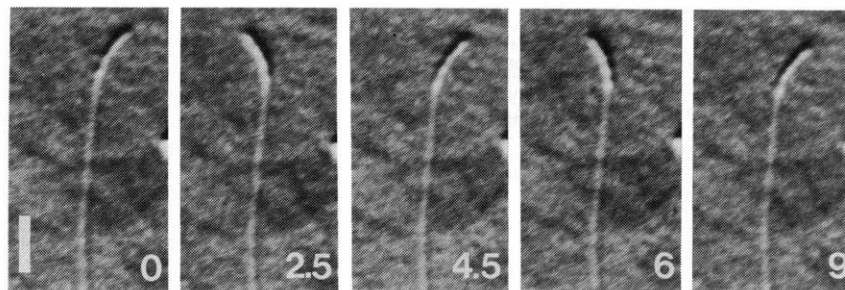
We have determined the direction of translocation of the *claret* motor protein using taxol-stabilized, asymmetrically labelled microtubules. In one set of experiments (Fig. 3), weakly fluorescent microtubules were selectively polymerized onto the plus

ends of brightly fluorescent, crosslinked microtubule seeds. In another set of experiments (Fig. 4) microtubules were selectively grown off the plus ends of fragments of sea urchin sperm flagellar axonemes. In either case, plus-end microtubules were selectively assembled by incubation of the seeds in an equimolar mixture of tubulin modified with *N*-ethylmaleimide and unmodified tubulin<sup>14–16</sup>. As seen with time-lapse fluorescence microscopy (in Fig. 3), the asymmetrical fluorescent microtubules move with their plus ends (dim fluorescence) leading and minus ends (bright fluorescence) trailing. The VE-DIC micrographs in Fig. 4 also show that the plus-ended microtubule leads the axoneme seed. As the *claret* motor protein is attached to the glass, these results demonstrate that the *claret* motor moves towards the microtubule's minus end, a direction characteristic of axonemal and cytoplasmic dyneins<sup>9,13,14</sup>, but opposite to that of kinesin, which is a plus-end translocator<sup>17</sup>.

The *claret* motor protein also has the unusual capability of generating torque, which produces microtubule rotation as the

FIG. 4 The *claret* motor protein produces microtubule rotation during translocation. Taxol-stabilized microtubules selectively assembled onto the plus ends of sea urchin axoneme fragments moved over the coverslip surface with the axoneme fragment trailing. Microtubules with trailing axoneme fragments moved at velocities ( $3.0 \pm 1.2$  μm min<sup>-1</sup>,  $n = 36$ ) similar to those of taxol-stabilized free microtubules (Fig. 2). Many axoneme fragments rotated as their attached microtubules moved over the coverslip surface. The frames show successive 180° rotations. Time in seconds is indicated on each frame. Scale bar, 2 μm.

**METHODS.** A mixture of 14 μM tubulin and 14 μM *N*-ethyl maleimide-modified tubulin was used to assemble microtubules selectively onto the plus ends of isolated *Lytechinus pictus* axoneme fragments<sup>14</sup>. Assembly



was carried out for 10–15 min at 37 °C. The resulting asymmetrical microtubule-axoneme complexes were diluted 20-fold into PM buffer containing taxol (final taxol concentration, 5 μM), and were used in the gliding assay (Fig. 2).



microtubule moves forward (Fig. 4). Rotation of the microtubules was most obvious for microtubules grown from fragments of outer doublets that had dissociated from the axonemes. The intrinsic curvature of these fragments provided an asymmetrical marker for the radial orientation of the microtubules<sup>14</sup>. The microtubules rotated in a clockwise direction as viewed from their minus ends. A complete rotation occurred every  $6.1 \pm 2.6$  s ( $n = 29$ ) of forward movement, and there were  $3.3 \pm 1.3$  rotations ( $n = 29$ ) for every 1  $\mu\text{m}$  of forward movement. Forward movement could continue even if rotation of the axoneme fragment was obstructed by the glass surface. These rotational features of the *claret* motor protein are strikingly similar to the rotation produced by 14S axonemal dynein, the only other microtubule motor protein reported to generate torque<sup>14</sup>. The mechanism of torque generation is unknown.

The *claret* motor protein was sensitive to inhibitors at concentrations known to inhibit cytoplasmic dynein activity<sup>8</sup>. Microtubule gliding, but not attachment, was blocked by 10  $\mu\text{M}$  vanadate, 100  $\mu\text{M}$  NEM (10 min treatment) or 2.5 mM of the non-hydrolysable ATP analogue AMP-PNP, but was unaffected by 1 mM AMP-PNP.

Why does a protein with 40–45% sequence identity to the kinesin motor domain have the directionality of a dynein ATPase? One possible explanation is that the *claret* motor can move bidirectionally and the bacterially expressed protein moves in only the minus-end direction. But its sensitivity to inhibitors and its ability to generate torque are more typical of dynein than of kinesin. Therefore, the most likely explanation is that the motor domains of kinesin and the dyneins are similar enough in sequence to allow molecular probes to the kinesin motor domain to identify both kinesin- and dynein-related motors. Like the *claret* motor, other members of the so-called kinesin superfamily<sup>18</sup> of motors, which have been identified by motor domain sequence similarity, may be minus-end directed motors.

The finding that the *claret* motor protein is a minus-end translocator suggests that it could be localized to the kinetochores on chromosomes and act to pull chromosomes polewards (toward the minus ends of microtubules) during prometaphase and/or anaphase. Unlike the rates reported for kinesin and cytoplasmic dynein<sup>8</sup>, the rate at which the *claret* motor protein translocates microtubules ( $4 \mu\text{m min}^{-1}$ ) is close to the rate of chromosome movement observed during most of mitosis and meiosis<sup>19</sup>. □

Received 19 September; accepted 10 October 1990.

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**ACKNOWLEDGEMENTS.** We thank A. Horne and V. Petrie for technical assistance, N. Gliksmann for discussions, W. Sale for axonemal dynein and G. Langford for squid kinesin. Taxol was supplied by the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute. Supported by the NIH (S.A.E. and E.D.S.). S.A.E. is a member of the Duke University Comprehensive Cancer Center.

## ERRATUM

### Identification of the 64K autoantigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase

Steinunn Baekkeskov, Henk-Jan Aanstoot, Stephan Christgau, Annette Reetz, Michele Solimena, Marilia Cascalho, Franco Folli, Hanne Richter-Olesen & Pietro De Camilli

*Nature* **347**, 151–156 (1990).

IN the published version of this article, the name of one of the authors (P. De C.) was incorrectly hyphenated. Indexing services are requested to use the correct form above.

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Articles should be accompanied by a heading of 50–80 words written to advertise their contents in general terms, to which editors will pay particular attention. A heading (printed in italic type) is not an abstract and should not usually contain numbers or measurements. The study should be introduced in more detail in the first two or three paragraphs, which should also briefly summarize its results and implications.

Articles may contain a few subheadings of two or three words. The meaning of the text should not depend on the subheadings, whose function is to break up the text and to point to what follows. There should be fewer than 50 references.

**Letters to Nature** are short reports of outstanding novel findings whose implications are general and important enough to be of interest to those outside the field. Letters should not have more than 1,000 words of text or more than four display items and should not occupy more than two pages of *Nature*. The first paragraph should describe, in not more than 150 words, the origins and chief conclusions of the study. Letters should not have subheadings or more than 30 references.

**Commentary** articles deal with issues in, or arising from, research that are also of interest to readers outside research. Some are commissioned, some are unsolicited. They are normally between one and four pages of *Nature* in length.

**News and Views** articles are intended to inform non-specialist readers about a recently published advance. Suggestions should be made to the News and Views Editor. Illustrations are welcome. Proposals for meeting reports should be agreed in advance.

**Scientific Correspondence** is for the discussion of scientific matters, including contributions published in *Nature*. Priority is given to contributions of less than 500 words and five references. Figures are welcome.

**Submission.** All manuscripts may be submitted to either London or Washington. They should not be addressed to editors by name. Manuscripts or proofs sent by air courier to London should be declared as 'manuscripts' and 'value \$5' to prevent the imposition of import duty and value-added tax (VAT).

All contributions submitted for publication in *Nature* should conform with these rules.

**Manuscripts** should be typed, double-spaced, with a good-quality printer, on one side of the paper only. Four copies are required, each accompanied by lettered artwork. Four copies of half-tones should be included. Reference lists, figure legends and so on should be on separate sheets, all of which should be double-spaced and numbered. Copies of relevant manuscripts in press or submitted for publication elsewhere should be included, clearly marked as such. Revised and resubmitted manuscripts should also be clearly marked as such and labelled with their reference numbers.

**Titles** should say what the paper is about with the minimum of technical terminology and in fewer than 80 characters. Authors should avoid active verbs, numerical values, abbreviations and punctuation and should include one or two key words for indexing purposes.

**Artwork** should be marked individually and clearly with the author's name, figure number and, when known, manuscript reference number. Original artwork should be unlettered. Ideally, no figure should be larger than 28 by 22 cm. Figures with several parts are permitted only if the parts are closely related, either experimentally or logically. Suggestions for cover illustrations, with captions, are welcome. Original artwork (and one copy of the manuscript) will be returned when a manuscript cannot be published.

**Colour Artwork.** A charge of £500 a page is made for 4-colour figures. Inability to meet these costs will not prevent the publication of essential colour figures if the circumstances are explained.

**Figure legends** must not exceed 300 words and ideally should be much shorter, and should use telegraphic form wherever possible. The figure should be described first, then, briefly, the method. Reference to a method described elsewhere is preferable to a full description. Methods should not be described in detail in the text.

**References** should be numbered sequentially as they appear in the text, followed by those in tables and finally those in the figure legends. Reference numbers apply only to papers published or in the press, and a different number should be given to each paper cited. All other forms of reference (including unreferenced abstracts) should be included in the text as personal communication, manuscript in preparation or preprint (with number and institution where appropriate). Text should not be included in the reference list. References should be abbreviated according to the *World List of Scientific Periodicals* (fourth edition, Butterworth, London, 1963–65). The first and last page numbers should be cited. Reference to books should clearly indicate the publisher and the date and place of publication.

**Abbreviations, symbols, units and Greek letters** should be identified the first time they are used. Acronyms should be avoided as much as possible and, when used, defined. Editors will shorten words if necessary. In case of doubt, SI units should be used.

**Footnotes** should not be used except for changed addresses or to identify the corresponding author if different from the first-named.

**Acknowledgements** should be brief. Grant numbers and contribution numbers are not allowed.

# Computer modelling at the single-neuron level

J.P. Miller

The computational capabilities of a single neuron depend to a large extent upon the structural and electrical properties of its dendritic branches. New modelling algorithms and a new generation of powerful UNIX workstations are being applied to the study of neuronal information processing.

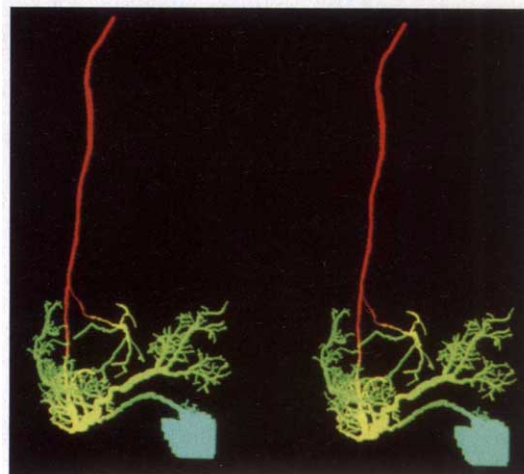
How does a nervous system encode, analyse and act upon the immense amount of information available from various sensory modalities and resident within its memory? Questions concerning the specific computational algorithms underlying neural information processing and the means by which those algorithms are implemented in real neural circuits continue to stimulate much research in the neurosciences. An increasing number of neuroscientists are formulating their working hypotheses concerning the operation of neural networks in the form of quantitative computer models capable of simulating patterns of electrical activity, which subsequently can be compared to actual neuronal activity observed in experiments. The 'computational units' in many of these network models are considered to be 'point integrator' neurons: isopotential compartments that calculate the linear sum of their inputs and set their output level according to some — possibly nonlinear — function of that sum. However, real neurons are much more complex than this, and the conclusions drawn from models developed using such simplified assumptions may be unrealistic. Model networks with much broader computational capabilities might be realized by incorporating more realistic and complex neural units.

First, the assumption of isopotentiality in response to synaptic input is rarely, if ever, valid, due to the complex dendritic branch structure characterizing most neurons<sup>1-3</sup>. Second, the demonstration of voltage-dependent conductance mechanisms on dendritic branches in many cell types invalidates conventional notions that synaptic currents are summed relatively linearly and then low-pass-filtered to the neuron's spike initiation zone or synaptic output site<sup>4</sup>. Third, the relationship between the transmembrane current at the spike initiation zone and the frequency of action potential generation can display nonlinearities that are much more complex than a 'simple' threshold behaviour<sup>5</sup>.

## How do neurons 'compute'?

A new generation of computer programs capable of simulating electrical current flow through the branches of very com-

Stereo pair image of a sensory interneuron in the cricket cercal sensory system, reconstructed with software developed at U.C. Berkeley. The voltage at each point is coded with colour, where pure red corresponds to +55 mV and turquoise corresponds to -70 mV. This is one timepoint in an animated sequence representing an action potential generated in response to synaptic input onto one dendritic branch. Voltages were calculated with the program 'NEMOSYS' on an IBM RS/6000 computer. Such images are typical of those that can be generated by most of the new generation simulation packages.



plex nerve cells is being developed and applied in many laboratories worldwide. Some of the general goals being pursued include: (1) a definition of the computations performed in neuronal dendrites, (2) a determination of the roles of nonlinear and time-dependent processes in these information-processing tasks, (3) a determination of the nature of cooperative interactions within and between aggregations of different types of voltage- and ligand-dependent membrane channels, and (4) a definition of the nature and functional significance of intrinsic stochasticity and threshold fluctuations in neuronal membrane potentials.

Two insights have recently emerged from such computer simulation studies which may be of considerable significance. First, the presence of voltage-dependent conductances on neuronal dendrites may allow a neuron to respond to independent synaptic inputs in a manner analogous to multiplication of those inputs<sup>6</sup>. Second, the presence of nonlinear conductances on dendritic spines (which are the morphological sites of synaptic contacts onto many types of neurons) may allow neural computations equivalent to extremely complex boolean logic operations<sup>7,8</sup>. In both cases, the spatial ranges over which independent inputs interact depend on the precise structural features of the dendritic branches and on the voltage-dependent electrical properties of the neuronal membrane.

## How do modellers compute?

What aspects of a neuron's structural and electrical properties are functionally relevant, and how can these complexities be incorporated into neuron models? An approach that offers many advantages from a computational standpoint, demonstrated by Rall as early as 1964<sup>9</sup>, is to formulate a model in which the neuron of interest is represented as a collection of short segments joined end-to-end in a 'tinker-toy' fashion to form a binary branched-tree structure. Each segment is chosen to be small enough to allow its representation as an isopotential compartment. The task of calculating the responses of a neuron to a distributed synaptic input is reduced to calculating the voltages at particular nodes between these compartments. Several reviews and examples of studies using this approach can be found in the book entitled *Methods in Neuronal Modelling*<sup>10</sup>. Chapter 13 documents the enormous range in the relative efficiency (that is, computational speed) of different numerical methods<sup>11</sup>. The choice of the appropriate computational method will depend upon the particular modelling problem at hand. For models of single nerve cells that have significant structural or biophysical complexity, there now seems to be a clear 'best choice' for the numerical computational scheme. This optimal computational scheme, recently recognized by M. Hines at Duke University<sup>12</sup>, represents a significant advance in the ability to model



the electrical voltage transients throughout the complex dendritic branches of neurons. The major advantage of this scheme is that the number of required computations scales linearly with the number of model compartments, whereas more conventional computational schemes scale as the square of the number of compartments.

Until recently, the use of compartmental computer models has been restricted to the few research groups where those models were developed. Times are changing: computer power is now relatively inexpensive, and the modelling software being developed and distributed from many laboratories is becoming more general purpose in nature, more interactive and more standardized in terms of the operating environment. The emerging standard, it seems, is to write the simulation software in 'C', to operate in the UNIX environment, and to utilize the 'X-windows' graphical interface. Programs written with these standards will run without significant modifications on machines ranging from IBM PS/2-70s to CRAYs. The most widely used computers are the mid-range 32-bit UNIX workstations, like the SUN workstations, the new IBM RS/6000s, DEC 'MicroVaxes', and the like.

Hines has incorporated his advanced computational schemes into a set of simulation packages ('CABLE' and 'NEURON')<sup>12,13</sup> which run on such machines. These computational schemes have also been incorporated into a simulation package called 'NEMOSYS' (see figure), which was developed in our laboratory at U.C. Berkeley by J. Tromp. They are also being incorporated into a third and very popular neural network simulation package called 'GENESIS', which was developed by J. Bower and his colleagues at Caltech. All of these programs (and perhaps several more of which the author is unaware) run in the UNIX/C/X-windows environment on a variety of 32-bit UNIX workstations, and are generally available to interested researchers.

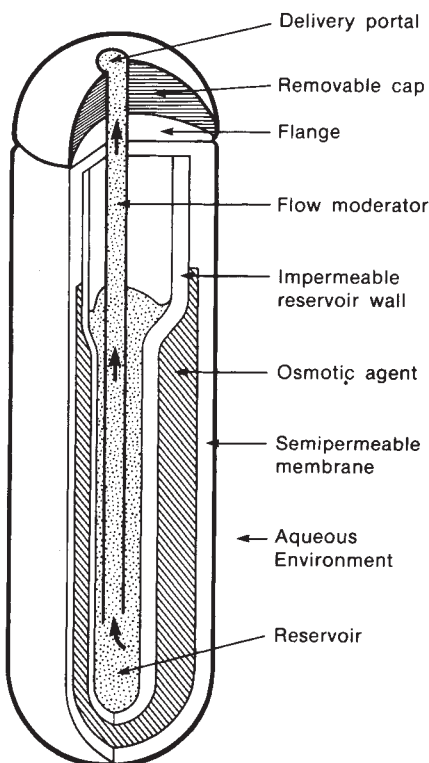
General purpose modelling packages such as these are likely to gain much wider use in neurobiology laboratories worldwide in the next few years. By using such programs to study how much of the structural and electrical complexity of a neuron's branching structure is essential to the fidelity and character of its operation, we may begin to understand the operation of nervous systems in terms of the structure, function and synaptic connectivity of the individual neurons from which they are constructed. □

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# Neuroscience now

Neuroscientists will convene in St Louis, Missouri, next week for the 20th Annual Meeting of the Society for Neuroscience. Product exhibits will include an implantable osmotic pump for controlled drug delivery, pocket-sized data loggers and monoclonal antibodies for detecting microtubule-associated proteins.

MEASURING just 3-cm long and weighing 1.1 grams, the ALZET Model 2001D 24-hour implantable osmotic pump from Alza can be used to study the effects of controlled drug delivery in research animals, such as mice and rats (*Reader Service No. 101*). The Model 2001D pumps at a rate of  $8 \mu\text{l h}^{-1}$  for 24 hours and is, therefore, useful for providing short-term, high-dose infusions or infusions of poorly soluble compounds. According to Alza, the continuous action of the osmotic pump allows the effects of agents with short half-lives to



Alza's 24-hour osmotic pump for controlled drug delivery.

develop fully and reproducibly. The osmotic pump may be of interest to researchers in the fields of neuroscience, immunology and cancer research, where it can be used for the one-day delivery of peptides, growth factors and other bioengineered molecules. The Model 2001D complements Alza's existing range of osmotic pumps, which are available for the delivery of drug solutions or suspensions at preprogrammed rates ranging from 24 hours to 4 weeks. Each pump costs \$16.70, when purchased in quantities of between 10 and 40. Alza will be in booths 616/618.

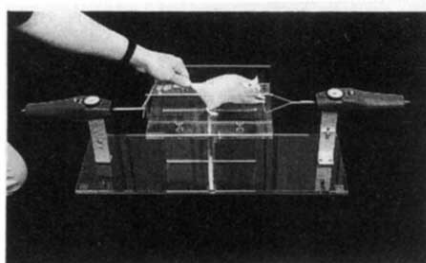
Researchers studying the electrophysiological properties of epithelial cells may be interested to hear that **ussing chambers** from World Precision Instruments are now available in autoclavable polysulphone as well as clear acrylic (*Reader Service No. 102*). The chambers are machined from solid blocks of material with eight entry ports for fluid lines, electrodes or agar bridges. Fluid compartments in each side of the chamber are separated by the epithelial membrane under study. The membrane is held in place by five sharp stainless steel pins located in one half of the chamber, which interlock with five holes in the opposite half. The eight ports are of the luer-type to provide leak-free attachment of the tubing and electrodes, says WPI. To prevent the formation of air bubbles in the chamber, voltage and current ports are recessed. Prices start at \$400 (US). WPI will be showing off its wares in booths 718/720/722.

Mini-Mitter, a manufacturer of physiological telemetry products, is introducing a new line of **ambulatory temper-**

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- Methods in Neuronal Modelling* (eds Koch, C. & Segev, I.) (MIT Press, Cambridge, Massachusetts, USA, 1989).
- Mascagni, M.V. in *Methods in Neuronal Modelling* (eds Koch, C. & Segev, I.) 439-459 (MIT Press, Cambridge, Massachusetts, USA, 1989).
- Hines, M. *Int. J. biomed. Comput.* **15**, 69-76 (1984).
- Hines, M. *Int. J. biomed. Comput.* **24**, 55-68 (1989).

ature and activity monitoring devices called Mini-Loggers (*Reader Service No. 103*). These pocket-sized, lightweight units feature batteries with lifetimes of 10 years or more, and have multichannel capabilities, 32,000 data-point RAM and PC-compatibility, says Mini-Mitter. Users can access the on-board microprocessor by plugging it into the serial port of any MS-DOS machine to set sample intervals and configure the available channels (up to eight on some models). Mini-Mitter's data loggers will be on display in booths 513/515.

Columbus Instruments, exhibiting in booths 652/654, has developed a **grip-strength meter** for mice and rats that can be used to provide an indication of



Take the stress and strain out of research with Columbus' grip-strength meter.

peripheral neuropathy (*Reader Service No. 104*). With stated applications in the areas of toxicology, pharmacology and neurophysiology, the \$2,490 (US) grip-strength meter has two push-pull autorecording strain gauges for measuring the maximum force applied by the forelimbs and hindlimbs of the test animal.

### Neurotoxin news

On display in booth 355 in St Louis will be the new line of **neurotoxins** from Bachem Bioscience (*Reader Service No. 105*). Included in the line-up are alpha, mu and omega conotoxins; conopressins; sarafotoxins; mast cell degranulating peptide; apamin; melittin; mastoparans; anthopleurin-A; charybdotoxins; and bungarotoxins. In addition, Bachem is offering isosteric and mono-substituted analogues of  $\omega$ -conotoxin, charybdotoxin and anthopleurin-A. All neurotoxins are analysed by HPLC and supplied with a data sheet stating peptide content, amino acid composition, purity and biological activity, says Bachem.

Research Biochemicals' line of ion channel modulators now includes a broad selection of **neurotoxins** (*Reader Service No. 106*). In particular, RBI offers apamin, charybdotoxin, dendrotoxin and MCD peptide which affect  $K^+$  channels, and  $\omega$ -conotoxin which is active at  $Ca^{2+}$  channels.  $\mu$ -conotoxin, which is reported to be active at both the  $K^+$  and  $Na^+$  channels, is also available. Completing the line-up is  $\beta$ -bungarotoxin, conotoxin MI and Joro spider toxin. Stop by RBI's booth, number 512, and receive a free copy of the company's 1991 wallchart which gives a full product listing, including details of pharmacological activity.

### Research tools: mAbs

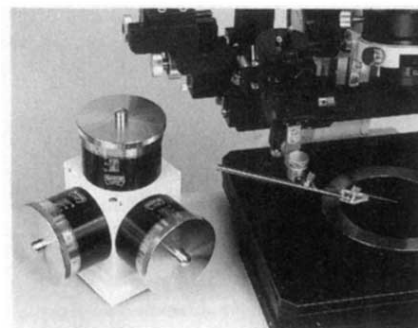
Bioproducts for Science is distributing a new panel of **monoclonal antibodies to human endothelium** from Serotec (*Reader Service No. 107*). MCA 547 identifies the CD34 epitope on haemopoietic cells. The mAb shows broad reactivity to human endothelium cells and some cross-reactivity with vascular-associated adventitia and some basement membranes. The mouse IgM monoclonal, MCA 639, reacts with human endothelium and has apparent specificity for kidney-associated endothelium, says BPS. Some stromal cross-

reactivity in other tissues has been noted. MCA 640 reacts with all samples of human endothelium that have been tested so far, and shows clean staining of endothelial luminal surfaces in decidual spiral arteries and capillaries. Similarly, MCA 641 also reacts with all human endothelium tested to date, and preliminary data suggest that this antibody recognizes thrombomodulin but does not inhibit thrombomodulin-dependent activation of protein C. All of the mAbs are tested for use in immunohistochemical staining of frozen tissue sections and cost \$292 (US) for 100 tests. BPS will be in booths 400/402.

The Biochemical Products Division of Boehringer Mannheim has introduced two new **monoclonal antibodies for detecting microtubule-associated proteins** (MAPs) in neuronal tissue — useful tools for studying neuronal growth, development and disease (*Reader Service No. 108*). Anti-MAP-2 and anti-tau can be used to identify the MAP-2 antigen in neuronal cell bodies and dendrites and the tau-1 antigen in axons. Both react with human, rat, avian and bovine brain tissue and have, says Boehringer, been tested for specificity, clean signals and reliability by immunohistochemistry and western blot analysis. See Boehringer in booths 320/322.

### Skilful manipulators

Visitors to booth 648 can see the new MO-303 **micromanipulator** from Narishige which is designed for precision micropositioning and, as such, is suitable for patch clamping applications (*Reader Service No. 109*). The hydraulically actuated \$4,938 (US) MO-303 headstage can be mounted



MO-303: micropositioning in 3-D.

on a variety of microscopes, providing ultrafine, three-dimensional movement via remotely located control knobs, 0.2-micron resolution and low drift ( $\pm 0.2$  micron over 2–3 hours), says Narishige. A remotely controlled hydraulic joystick model — the MO-302 — is also available.

According to Newport Bio-Instruments, smooth motion and a rapid response to hand adjustments are features of the MX500 series of **hydraulic micromanipulators**, making them suitable for long-

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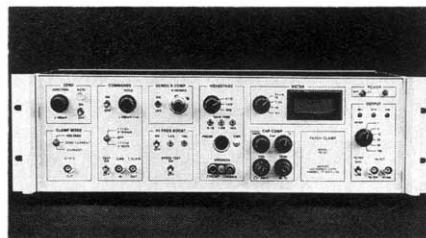
Reader Service No.56

term intracellular and patch clamp recordings, ultrafine cell probing and micro-injection applications (*Reader Service No. 110*). Instruments in the MX500 series can be attached to most coarse manipulators. However, the instrument's compact design also allows the micromanipulators to be mounted directly to either an upright or inverted microscope. Direct mounting onto a microscope stage eliminates the relative motion between the microscope and the manipulator, says NBI. This arrangement enables focusing during viewing by moving the stage and micromanipulator together as a whole unit. Two models are available: the \$1,450 (US) one-axis MX510 and the \$2,950 (US) three-axis MX530. Booths 906/908 will feature exhibits from NBI.

### Patch clamping paraphernalia

Medical Systems has introduced the Model VC-1200 unit for studies involving **two-electrode voltage clamping**, where one electrode is used to record membrane potentials and the other passes the clamping current (*Reader Service No. 111*). Designed with a wide current and voltage capacity, the \$2,265 (US) VC-1200 has, says the company, the speed, amplification and flexibility to clamp small cells as well as large cells, such as frog oocytes. The two-electrode voltage clamp unit comes with an automatic overload cut-out to protect cells, a 'stimulus' mode for use with iontophoretic stimulation, a two-pole adjustable bandpass Bessel filter, virtual/chassis ground options and phase controls. See booths 638/640/642.

For single channel and whole cell patch-clamp recordings, Warner Instruments recommends its PC-501 **patch and whole cell clamp**, which sells for under \$2,500 (US) (*Reader Service No. 112*). Three small, lightweight probes are available, each mounted in a micropositioner: the 10 GΩ feedback resistor for single channel



Patch/whole cell clamp — see booth 326.

recording, 1 GΩ resistor for voltage clamping of small cells, and the 100 MΩ resistor for voltage clamping of larger cells. The instrument is equipped with a built-in test generator, active low-pass filter, auto zero and variable output gain. The gain telegraph output permits computer monitoring of the output signal. Warner will be in booth 326.

The latest version of PhoCal — a system for the real-time measurement of living

cells from Joyce-Loebl — is now available for both dual-emission and dual-excitation fluorochromes (*Reader Service No. 113*). Uses of PhoCal include the online analysis of intracellular ionic concentration, and the simultaneous recording of patch clamp current and voltage, or extracellular pH, or muscle tension. PhoCal now incorporates two new features: dual-channel photon counting for improved



PhoCal system upgrade from Joyce Loebel. signal-to-noise ratios and Joyce-Loebl's rotating filter wheel and controller for dual-excitation fluorochromes. The PC-based system is compatible with various standard laboratory interfaces and microscopes and will accept any amplifier or transducer that produces a voltage or current. Interested researchers can evaluate the product by obtaining a demonstration floppy disk from the company, which is supplied with data files taken from experiments using both dual-excitation and dual-emission fluorochromes.

### What's new

For up-to-date information on new **venoms and toxins** from Accurate Chemical and Scientific, see the company's latest product catalogue (*Reader Service No. 114*). The catalogue lists over 25 new products, including snake venoms, scorpion venoms, batrachian venom and venoms from other arthropods such as hymenoptera, centipedes and spiders. Scorpion and spider venom kits and the purified toxins ammodytoxin-A, argiotoxin and MCD peptide are also available.

The 1990-1991 Physiology Research Instruments **catalogue** from Stoelting lists many new additions to existing lines of micromanipulators, stereotaxic instruments, pipette pullers and microforgers, and should be of interest to researchers in the fields of physiology, pharmacology and neuroscience (*Reader Service No. 115*). The catalogue features new animal assessment instruments, including a non-destructive body composition analysis instrument. Stop by Stoelting's booths — 820/822.

*These notes are compiled by Diane Gershon from information provided by the manufacturers. To obtain further details, use the reader service card bound inside the journal. Prices quoted are sometimes nominal, and apply only within the country indicated.*